CHARACTERIZATION OF THE PREVALENCE AND FECAL EGG COUNT INTENSITY OF GASTROINTESTINAL NEMATODES AND THEIR MANAGEMENT IN WESTERN CANADIAN COW-CALF HERDS

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Saskatoon

By
Felicity Kaye Wills

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ABSTRACT

Gastrointestinal nematodes (GIN) have a negative impact on animal health and production in grazed beef cattle. The impact that GIN have on cattle is dependent on the interaction of many factors including the biology between the host (cattle) and the parasite, climate, cattle management/husbandry, pasture/stock management and varies between geographical locations, herd to herd and animal to animal. There is a scarcity of current information regarding GIN epidemiology and management specific to western Canadian beef cow-calf herds, an important sector of the agrarian economy. Therefore, the overarching objective of this thesis was to provide current information about the epidemiology and management of GIN in beef cow-calf herds of western Canada. This objective was examined in three parts: i) determining the prevalence and FEC intensity of gastrointestinal nematode burdens in different production types (cows, calves and replacement heifers), ii) characterizing the herd-level gastrointestinal nematode burden of heifers quantitatively and qualitatively by conducting fecal egg counts and determination of nematode larval species identity and iii) characterizing the current management strategies employed by producers in the control of gastrointestinal nematodes by producers of the western Canadian beef cow-calf industry. In study 1, fecal egg counts (FEC) provided by Merck Animal Health Canada from cows (n=1,780), calves (n=980) and replacement heifers (n=960) from 201 herds over 2012, 2013 and 2014 were analyze using generalized estimating equations (GEE) (STATA®14) for the prevalence and mean eggs per gram (EPG) for Trichostrongylid-type eggs, Nematodirus spp. and Trichuris spp. The prevalence of Trichostrongylid-type eggs was uniformly high across all production types (78; 95% CI 75-82), while the mean EPG was consistently low (4.9; 95% CI 3.9-5.9). *Nematodirus* spp. egg positive samples came most frequently from calves, with an appreciably high predicted prevalence of 36% (95% CI 30-42). *Trichuris* spp. eggs were a very infrequent finding at an overall prevalence of 0.2% (7/3891; 95% CI 0.08 – 0.4). Study two used the same methodology as study 1. However, samples utilized were form 1,655 heifers (n=85 herds) enrolled in a pilot disease surveillance network (Western Canadian Cow-Calf Surveillance Network (WCCCSN)). The prevalence (95% CI) of Trichostrongylid-type egg positive samples was high at 92% (95% CI 89-95). The prevalence of *Nematodirus* spp. (2%; 95% CI 1-3) and *Trichuris* spp. (1%; 95% CI 1-2) was very low.
The level of FEC intensity was consistently low, with a mean EPG (95% CI) of Trichostrongylid-type eggs was 5.0 (95& CI 4.4– 5.9). Herds with >300 cow-calf pairs had a significantly ($p<0.01$)

lower mean predicted Trichostrongylid-type EPG at 5.0 EPG (95% CI 3.6-6.5) compared to herds with ≤300 cow-calf pairs (5.3 EPG; 95% CI 4.4-6.2). For Study 3 in May 2016, 105 questionnaires were administered to producers enrolled in the WCCCSN pilot disease surveillance network described in study 2, to describe the current management strategies utilized by these herds to control GIN. Ninety-seven of the administered questionnaires were returned, for a response rate of 93%. The responses from the producers revealed the almost uniform dependence of producers on the use of a pour-on macrocyclic lactone parasite control product 96% (92/96; 95% CI 89-98) in the fall as part of a routine farm management program as the method of choice for the treatment of GIN in western Canadian beef cow-calf herds. The combined results of these studies reveal a high prevalence but low level of FEC intensity of GIN in western Canadian beef cow-calf herds across all animal production types. The results of the management questionnaire raise concerns about the impact that current management strategies may have on the development of anthelmintic resistance in GIN populations. This paired with the consistently high prevalence of GIN seen highlights the need for continuing research into the epidemiology, diagnosis and management of GIN in beef cow-calf herds of western Canada.

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LIST OF ABBREVIATIONS

AB	Alberta
ADG	Average daily gain
AR	Anthelmintic resistance
BCS	Body condition score
CAD	Canadian dollars
C. oncophora	*Cooperia oncophora*
C. punctata	*Cooperia punctata*
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram
EP3G	Eggs per three grams
FEC	Fecal egg count
FECRT	Fecal egg count reduction test
FSG	First season grazing
GIN	Gastrointestinal nematode
L3	Infective third stage larvae
MB	Manitoba
NAHMS	National Animal Health Monitoring System
OD	Optical Density
ODR	Optical density ratio
O. ostertagi	*Ostertagia ostertagi*
PCR	Polymerase chain reaction
PGE	Parasitic gastroenteritis

rDNA	ribosomal Deoxyribonucleic acid
RCF	Relative centrifugal force
rpm	Rotations per minute
SK	Saskatchewan
spp.	Species
TT	Targeted Treatments
TST	Targeted Selective Treatment
US	United States
USDA	United States Department of Agriculture
WAAVP	World Association for the Advancement of Veterinary Parasitology

CHAPTER ONE
LITERATURE REVIEW

1.1 INTRODUCTION

Efficient ruminant livestock production will be crucial in meeting the meat demands of a world with an increasing population and changing climates. All grazing ruminants are exposed to gastrointestinal nematodes (GIN) while on pasture (Charlier et al., 2014). Determining the exact impact that GIN burdens have on cattle can be difficult to quantify as it is influenced by many factors including cattle health/immunity, the relationship between the cattle and the GIN, climate, grazing management and herd husbandry (Charlier et al., 2014; Vercruysse and Claerebout, 2001).

Although it is challenging to estimate the exact cost of GIN burdens because of the difficulty of quantifying subtle production losses, they are considered among one of the most costly diseases in the cattle industry (Charlier et al., 2014; Corwin, 1997). Conservative estimates reported are that GIN parasites cost the American cattle industry more than $2 billion per year in lost productivity and increased operating expenses (Stromberg and Gasbarre, 2006). This estimate is based on the assumption that most cattle in the United States receive on average slightly more than one anthelmintic treatment per year, resulting in annual expenditures of almost $500 million, with losses in productivity accounting for the remaining costs (Stromberg and Gasbarre, 2006).

Clinical symptoms of GIN parasitism such as weight loss, submandibular edema and diarrhea are referred to as parasitic gastroenteritis (PGE) (Anderson et al., 1965; Hawkins, 1993). Since the development of effective and inexpensive anthelmintic drugs, clinical disease caused by GIN is not commonly seen any longer in North America. Subclinical disease in the form of production losses now have the biggest impact on the profitability of cow-calf herds (Eysker and Ploeger, 2000). However there has been an increase in the strength and reported occurrence of disease caused by GIN (van Dijk et al., 2010).

Along with the reduction in clinical PGE seen because of the widespread use of routine 'blanket' application of anthelmintic drugs by beef cattle operators, anthelmintic resistance (AR) is also an emerging threat. The increasing reports of AR in cattle highlight the need to develop more sustainable GIN control programs that do not rely heavily on the routine blanket use of anthelmintic drugs (Höglund et al., 2009; Kenyon and Jackson, 2012). If more sustainable GIN control programs are not developed, GIN control will depend on the pharmaceutical industry to develop new products faster than the emergence of AR (Gasbarre, 2014).

Gastrointestinal nematode burdens vary among geographic locations, seasons, years, herds and individuals. Some of this variability is because of the differing survivability of the infective stages of GIN on pasture, because of many factors including, the differing ideal environmental conditions (temperature, humidity, accumulated precipitation) of different GIN species (Beck et al., 2015; Wilson et al., 2001). A successful anthelmintic strategy has to target the specific GIN species or mix causing harm to the cattle; it has to have nominal environmental impact and accommodate different climate and farm management practices and geographical locations. Therefore, understanding the epidemiology of GIN in a specific region is critical in developing efficient and effective control programs (Waller, 2006).

This chapter will discuss the GIN of importance to the western Canadian beef cow-calf industry. In particular it will focus on the epidemiology and the challenges related to the quantitative diagnosis of these GIN in pasture grazed beef cattle, as they relate to the information that is required to be able to create more effective GIN management programs that allow for efficient production while limiting the development of AR.

1.2 Gastrointestinal nematodes of importance in beef cattle

Gastrointestinal nematodes are from the phylum Nematoda, also known as roundworms. In temperate regions, *Ostertagia ostertagi* and *Cooperia oncophora* species are considered to be the most important internal parasites of beef cattle (Ranjan et al., 1992; Walker et al., 2013). In the United States' National Health Monitoring System's 2007-2008 beef study, from 20 fecal samples from each of 99 herds, 91% contained *Cooperia*, 86% *Nematodirus* and 79% *Ostertagia* (USDA, 2010). Both *O. ostertagi* and *C. oncophora* belong to the superfamily *Trichostrongyloidea*. Other related genera of this superfamily that commonly affect cattle are

Nematodirus spp., *Trichostrongylus* spp. and *Haemonchus* spp. Mixed burdens are most commonly found in grazing cattle (Avramenko et al., 2015).

1.2.1 Ostertagia species

Ostertagia ostertagi is considered one of the most important species of GIN of beef cattle, as it is one of the most pathogenic. *Ostertagia ostertagi* is particularly important in the temperate climates of North America as the parasite is well adapted to cooler conditions and survives well over winter on pasture, in soil, or in an arrested larval state (known as hypobiosis) within cattle (Stromberg and Averbeck, 1999). The primary site of infection for *O. ostertagi* in cattle is the abomasum. Adult parasites are slender, brownish-red worms reaching approximately 1cm in length; females are considered to have a low fecundity, laying approximately fifty eggs per day (Scott and Sutherland, 2009).

Clinical disease because of *O. ostertagi* is most frequently seen in young stock. Protective immunity to *O. ostertagi* develops slowly in cattle when compared to other GIN species, also contributing to its pathogenicity (Claerebout and Vercruysse, 2000). Two types of clinical disease caused by *O. ostertagi* are described in the literature. Type I ostertagiasis is a disease of young susceptible cattle in the summer and fall months (northern climate). Infective larvae after being consumed with pasture mature in the glands of the abomasum to become adults, in approximately 21 days. These young adult worms then break out of the glands of the abomasum, thereby creating substantial damage. Depending on the intensity of burden, clinical signs may vary from reduced growth/production because of reduced appetite to clinical ostertagiasis, which includes generally poor condition, a rough hair coat, profuse diarrhea, rapid weight loss, sub-mandibular edema, anemia and mortality (Myers and Taylor, 1989).

Type II ostertagiasis occurs in yearling or mature cattle. After cattle have acquired large numbers of larvae that undergo hypobiosis, type II disease occurs when arrested larvae begin the remainder of their maturation process. In northern climates, this maturation and subsequent eruption occurs during early spring. The clinical signs are the same as type I ostertagiasis. If emergence of parasites from the abomasal glands is gradual, a protracted disease occurs; if a substantial maturation occurs over a short period, the clinical signs are severe and can result in mortality, even in adult cattle. In less severely infected animals appetite suppression and production losses may be the only signs of burden (Myers and Taylor, 1989).

Clinical disease caused by *O. ostertagi* is now rarely seen in North America; the most economically important effect of *O. ostertagi*, like with most GIN, is the loss of appetite and, therefore reduced growth rate in the affected cattle (Stromberg and Gasbarre, 2006).

1.2.2 COOPERIA SPECIES

In a 2008 USDA (United State Department of Agriculture) NAHMS (National Animal Health Monitoring System) survey of United States (US) cow-calf herds, *Cooperia* spp. were the most prevalent parasites (Stromberg et al., 2015; USDA, 2010). *Cooperia* spp. is considered mildly pathogenic. Their primary site of infection is the small intestine and adult worms are 6-9 mm long, light red and coiled in appearance. *Cooperia* spp. is highly fecund and largely contributes to high fecal egg counts seen in young stock during their first grazing season. *Cooperia* spp. are usually seen as part of a co-burden with *O. ostertagi*. Cattle mount a rapid immune response to *Cooperia* spp. when compared to other GIN such as *O. ostertagi* and, consequently, both intestinal burdens and fecal egg counts decline towards the end of the first grazing season (Armour et al., 1987). *Cooperia* spp. causes damage to the small intestine that results in inappetence, slow growth, lowered nitrogen retention and loss of plasma proteins into the gut (Armour et al., 1987). Production losses are the most significant effect of *Cooperia* spp. burdens. In a 2012 study, Stromberg et al. found a decrease of 0.24 lb. (0.11 kg) of average daily gain for calves infected with a monoculture of *C. punctata*, a 7.4% decrease when compared to uninfected calves (Stromberg et al., 2012).

The 2007-2008 NAHMS survey also found increasing AR particularly to pour-on macrocyclic lactones in *Cooperia* spp. in the US (Gasbarre, 2014). Historically, the most common species of *Cooperia* seen in cattle is *C. oncophora*. The development of monoculture burdens of GIN in cattle is unusual, but monocultures of *C. punctata* have been diagnosed in the US, and this shift may be because of the use of routine blanket treatments of anthelmintics and the increase of multi-drug resistant parasites (Gasbarre et al., 2009a, 2009b). The recent change in the epidemiology of GIN in US cow-calf beef herds, with the emergence of *C. punctata* monocultures reinforces the need to assess the current epidemiology of GIN in beef cattle in western Canada.

1.3 EPIDEMIOLOGY

Understanding the epidemiology of GIN in cattle is essential to the development of strategic parasite control programs. Control strategies should be aimed at controlling both the adult worm burden within cattle, as well as the pasture larval populations. Furthermore, control approaches should be aimed at limiting the development of AR (Sutherland and Leathwick, 2011). It is important to know when the peak transmission times are in a given geographic location and under certain management styles if a successful control program is to be designed (Stromberg and Averbeck, 1999).

From the studies that have been conducted in Canada and the Northern United States, generally utilizing fecal egg counts (FEC) as the measure of infection prevalence and intensity, a general assumption of the pattern of GIN epidemiology has been adopted. Ranjan (1992), described that mature cows generally had low FEC year round; however, FEC were lower in winter and peaked in spring (May to early June), coinciding with turnout. Following spring turn out to pasture the immunologically naïve calves begin to ingest infective L3 larvae, that have overwintered (Falzon et al., 2014; Gibbs, 1988; Slocombe and Curtis, 1989), cows also contribute to the pasture contamination. The importance of each of these methods of infection must be elucidated for cattle of the western Canadian prairie provinces. The pre-patent period of most GIN during the warm summer months is approximately 21 days and so during the ensuring spring and summer there is a magnification of the number of infective larvae on pasture, resulting in calves' FEC continuing to increase until late October to December, at which time most of the ingested larvae arrest development (hypobiosis) and cease egg production. This arrested development, along with housing which reduces the risk of transmission, may result in low FEC in both cows and calves over winter.

In Canada, the increase in beef cow-calf herd size, management and factors such as later spring calving, more intensive grazing practices, and changing climate have been identified as potential risk factors that may alter the epidemiology of GIN burdens in beef cow-calf herds (Beaulieu, 2015; Fox et al., 2015; Gethings et al., 2015; Jelinski et al., 2016, 2015; Stromberg and Averbeck, 1999; Yazwinski and Tucker, 2006). These changing risks necessitate a current study of the GIN epidemiology specific to beef cattle in western Canada. There is little recent literature related to this subject published and those studies available are very specific to location, animal type and production system. Colwell et al (2014) reported on the annual variation in

serum antibody concentrations to Ostertagia ostertagi in beef weaners pastured in southern Alberta. Also Jelinski et al (2016) conducted a survey of gastrointestinal parasites in 14 Saskatchewan beef herd during summer 2014, in which they found that the prevalence and FEC burden intensities of gastrointestinal nematode parasites in cow-calf herds in Saskatchewan were comparable to what is seen in cattle grazing in the northern regions of the United States.

1.3.1 Life cycle of gastrointestinal nematodes

Gastrointestinal nematodes are single-host parasites that rely on their host (e.g. cow or calf) as well as their environment (i.e. pasture) to complete their direct life cycle. When infecting the host, the parasitic stages (i.e. fourth stage larvae and adults) create lesions in the gastrointestinal tract, altering its physiology and negatively impacting the animal's performance and welfare (Scott and Sutherland, 2009).

The life cycle of GIN involves both a free-living phase in which development occurs on the pasture outside of the host and a parasitic phase which occurs within the host after the infective third-stage larvae is ingested with forage. Figure 1.1 outlines the basic life cycle of GIN in cattle.

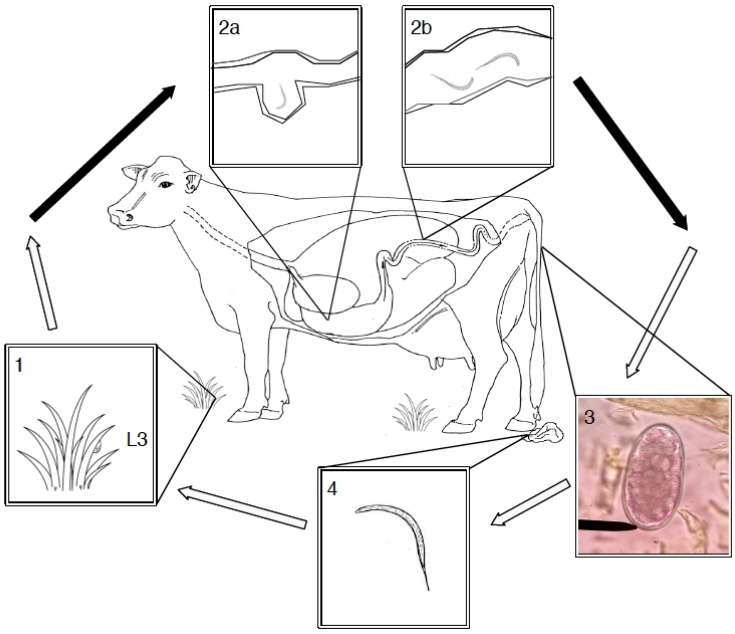

Figure 1.1 The life cycle of GIN in cattle. The unfilled arrows represent the free-living phase of the life cycle and the filled arrows represent the parasitic phase of the life cycle. Once ingested, the site of predilection is the abomasum(2a) and the small intestine(2b) for O. *ostertagi* and C. *oncophora*, respectively.

1.3.2 FREE-LIVING PHASE

The free-living phase of the direct life cycle begins when eggs from infected cattle are passed through feces into the environment (onto pasture; Figure 1.1, Image 3). These eggs contain first-stage larvae. Within the fecal pat, this larvae molts twice to become infective third-stage larvae (Figure 1.1, Images 1 and 4). This third-stage larvae is capable of infecting a host and is also known as the infective third-stage larvae, L3 (Scott and Sutherland, 2009). The process of development from egg to infective L3 in the free-living phase is dependent on environmental influences, particularly temperature and moisture (Pietrock and Marcogliese, 2003). The optimal temperature for development is between 23°C and 25°C and the process can take as little as 10 days. At temperatures above 32 °C desiccation of larvae ensues and below 6°C very little development occurs (Bryan and Kerr, 1989). Water is also essential for the survival of larvae during the free-living phase. Firstly, moisture is necessary for movement of larvae through the fecal pat and secondly, once an egg has developed into an infective L3 moisture is also necessary for the migration of the infective larvae onto vegetation where it can be consumed by the cow (Scott and Sutherland, 2009). Grazing cattle are infected when they ingest infective larvae from the pasture. In the early part of the grazing season, these larvae may be the survivors of the previous season's parasite population that have over-wintered on the pasture. Later in the season, the larvae on the grass originate from eggs that have been passed from infected cattle during the season.

A recent study conducted in Alberta, Canada, looked at spatial and temporal variability of GIN transmission among calves across the province (Beck et al., 2015). The study found that humidity, air temperature, and accumulated precipitation were significant predictors of the risk of GIN transmission in this northern climate. Slocombe (1973) also found that many of the GIN parasites, in the L3 stage, could overwinter successfully on pasture in Ontario, Canada. A more recent study conducted by Falzone et al. (2014) showed that larvae from the *Trichostrongylus* spp. and *Nematodirus* spp. could overwinter on pasture in Ontario and still infect sheep the

following spring. Whether larvae are also able to survive on pastures to infect cattle of the western Canadian prairie provinces, which are historically colder and drier (Government of Canada, 2017) has not been specifically elucidated.

1.3.3 Parasitic phase

The parasitic phase of the direct life cycle begins once the cow consumes the infective third-stage larvae when grazing pasture. In the cow, the infective L3 exsheath and migrate to their preferred site of infection. The infective larvae subsequently molt twice to become fourth-stage and fifth-stage larvae and then molt a final time to become mature adults that mate to produce eggs which are passed into the environment with feces (Scott and Sutherland, 2009). There are many factors that contribute to the level of exposure of grazing cattle to parasitic stages of GIN, including grazing patterns, stocking density, climate and animal age/immunity. These factors all affect the four main drivers of the number of infective GIN on pastures. The four key elements of infection pressure are larval establishment rate, hypobiosis rate, adult GIN mortality rate and fecundity (Verschave et al., 2014). These factors often also contribute to a GIN pathogenicity. An important aspect of the life cycle of GIN - in particular *O. ostertagi* - is the 'arrested development' or hypobiosis of fourth-stage larvae. The stimuli behind this hypobiosis is not fully understood; evidence suggests that cow immunity, nematode population size and seasonal conditioning of infective larvae are important factors (Eysker, 1997; Fernández et al., 1999). Resumption of development usually occurs after several months when environmental conditions become favorable for survival of eggs in the environment. In temperate northern climates, this is generally in late winter to early spring (Gibbs, 1993).

1.4 Impacts on production

Gastrointestinal nematode burdens, whether clinical or subclinical, have a negative effect on performance, health and welfare of the cow or calf, leading to negative economic implications for operators (Reinhardt et al., 2006). The reported impacts of GIN burdens on cattle make GIN one of the most costly diseases in the beef industry (Lawrence and Ibarburu, 2006; Stromberg and Gasbarre, 2006). There are a multitude of physiological changes that occur in cattle infected with GIN that ultimately result in decreased appetite, impaired gastrointestinal function, changes

in protein, energy and mineral metabolism, and alterations in water balance (Fox, 1997). Physiological changes are a result of several factors including damage to the gastrointestinal tract and the cattle's immune response to the parasites. Gastrointestinal parasitism can also affect the animal's immune response, decreasing its ability to respond to other burdens (Stromberg and Gasbarre, 2006). Reduced feed intake because of the suppression of appetite is perhaps of most importance economically as it results in decreased weight gain and/or weight loss (Stromberg and Gasbarre, 2006).

The impact on commonly used methods of production measurement, such as average daily weight gain (ADG), associated with the use of an anthelmintic drug on pastured cow-calf pairs, stocker calves and feedlot entrants, in climates where gastrointestinal nematode larvae are hypothesized or known to overwinter on pasture are summarized in Appendix A1.1. The results of these studies show that there is evidence that treatment with an anthelmintic drug has an effect on ADG in beef cattle production systems in northern climates. There is also evidence that the use of anthelmintic drug treatments also improved reproductive efficiency on cow-calf herds in these same climates (Larson et al., 1995; Stromberg et al., 1997). It is important to note however that these studies are very specific to locations with differing climates, resulting in different GIN epidemiology than western Canada. The studies are also very specific to a certain treatment protocol and production system and thus the results of these studies are difficult to directly compare to what may be found in western Canadian cow-calf herds.

First season grazing (FSG) calves on pasture are most at risk of acquiring heavy burdens of GIN, because of their lack of immunity. The epidemiology of GIN in adult cows however must also be closely considered as they act as a source of pasture contamination, which is the main source of GIN infective larvae for FSG calves. Heavy burdens of GIN, potentially containing anthelmintic resistant parasites, in weaned calves from cow-calf herds are particularly important as many of these calves directly enter feedlots where their performance may be significantly affected (Jim et al., 1992; MacGregor et al., 2001; Reinhardt et al., 2006). Another important consideration in the effective control of GIN burden in calves entering feedlots from cow-calf herds is the potential for reduced efficacy of vaccines and, therefore, increased susceptibility to other diseases such as bovine respiratory disease (Gasbarre, 1997). The relationship between GIN burdens and the immune response to vaccination has not been clearly defined and studies conducted to date have yielded mixed results (Charlier et al., 2013; Yang et

al., 1993a, 1993b).

1.5 Diagnosis

Eysker & Ploeger (2000), recommended the integration of GIN monitoring into herd-health and husbandry programs of cow-calf herds. The authors suggested a checklist of factors that a diagnostic test should fulfill in order to be most beneficial for herd level GIN surveillance. That check list included (1) the test enables an estimate of exposure; (2) the test values should reflect production losses; (3) the test values can be used to predict the risk of future production losses and allow recommendation or appropriate preventative measures; (4) the test results are easy to assess and (5) the test is inexpensive. The authors highlighted the need for the test to be quantitative because GIN burdens are ubiquitous. All these factors must be considered when evaluating characteristics of tests used for the diagnosis of GIN in cattle.

The ability to identify cattle that will respond favorably to anthelmintic treatment in the case of a subclinical GIN burden would be desirable in the cow-calf industry in order to reduce routine blanket treatment of all stock (Charlier et al., 2014; Sanchez et al., 2002). Although clearly defining producers' reasons behind their choice in treatment with an anthelmintic drug is important, as other treatment considerations such as the treatment of ectoparasites such as lice may strongly impact the ability to alter treatment strategies for GIN in beef cow-calf herds of western Canada.

1.5.1 Fecal egg counts

Historically, fecal egg counts (FEC) have been the most frequently used method to estimate GIN burdens in cattle, both clinically and in the research setting. This may be because of the inexpensive and highly accessible technology of this diagnostic test. There are several different methodologies for performing FEC in cattle, each of which has advantages and disadvantages (Levecke et al., 2012b). The different techniques for conducting FEC have different minimum detection limits, which is the lowest number of eggs per gram (EPG) of feces that can be detected by the given technique. Considering the minimum detection limit is particularly important when assessing FEC techniques used for adult cattle that routinely have

low FEC and when comparing results of different studies (Larsson et al., 2010; Levecke et al., 2012b, 2011).

Perhaps the simplest FEC technique is the McMaster technique. Its minimum detection limit is only 10 to 50 eggs per gram of feces; this is one of the reasons it is not routinely used in cattle as they frequently have a FEC lower than this (Burrows et al., 1980). One of the most commonly used technique for FEC in cattle is the modified Wisconsin centrifugal flotation technique, which is based on flotation of eggs in a sugar solution in a centrifuge tube, whereby eggs are recovered by means of adding a cover slide to the meniscus of the flotation solution (Levecke et al., 2012b). This technique is particularly useful in cattle as the minimum detection limit is 1-2 EPG (Egwang and Slocombe, 1981; Levecke et al., 2012b).

Fecal egg counts are commonly performed on fecal samples from cattle to gauge the level of GIN burden and/or to determine treatment efficacy in a herd. Fecal egg counts are perhaps most effectively used in first season grazing (FSG) cattle, when the results of the FEC relate best to infective larvae ingestion (Eysker and Ploeger, 2000). It was suggested by Vercruysse and Claerebout (2001), that if a group of FSG cattle have a mean FEC greater than 200 EPG five to ten weeks after turn out, then the likelihood of parasitic gastroenteritis increases and anthelmintic treatment is warranted. Thus, 200 EPG is commonly used as a threshold for treatment in FSG cattle to prevent clinical disease. However, it is presently unknown at what level of egg shedding subclinical production losses are occurring (Vercruysse and Claerebout, 2001).

Although FEC have been extensively used in the diagnosis of GIN infection in cattle, they have limitations that hamper their ability to accurately quantify and describe the epidemiology of the GIN burdens. Firstly, FEC only reflect the egg output of adult female worms at the time of sampling. This does not take into account other factors such as immature or encysted stages or the fecundity of different GIN. Also, while FEC are well correlated with actual burden levels in the first half of the season in FSG cattle (Eysker and Ploeger, 2000), the development of acquired immunity to these parasites during the first grazing season reduces the correlation between FEC and GIN burden because of a reduction in egg production by adult GIN. For the same reason there is poor correlation between FEC and actual worm burden in mature cattle (Michel, 1969).

Other factors that limit the usefulness of FEC as a diagnostic technique for GIN burden include the clustering/aggregation of GIN burdens within groups of cattle whereby the minority of cattle harbor the majority of parasites (Denwood et al., 2012; Morgan et al., 2005). Gasbarre et

al. (1996) advocated that in order to be informative about the herd-level parasite burden, a fecal sample for FEC analysis should contain a minimum of 20 individual fecal samples from cattle in that herd, because of this over-dispersion. Additionally, FEC show low repeatability, particularly when the number of eggs per gram is low and only a small volume of feces is analysed (Gasbarre et al., 1996).

1.5.2 Other diagnostic techniques

Selecting cows or calves that will have a positive response to treatment with an anthelmintic drug, relies on identifying thresholds for treatment (Sanchez et al., 2002). To date, an egg per gram (EPG) of feces threshold that differentiates between subclinically infected cows or calves that would benefit from treatment has not been established. Eysker and Ploeger (2000) suggested that two promising diagnostic alternatives to the FEC to establish treatment thresholds are serum pepsinogen levels and immunological assessment (ELISA) of antibody titers.

1.5.2.1 Serum pepsinogen

Serum pepsinogen concentrations have primarily been used for diagnosis of *O. ostertagi* burden in cattle (Berghen et al., 1993; Michel et al., 1978; Mylrea and Hotson, 1969). In the first grazing season, high pepsinogen values in calves correlate with the occurrence of PGE (Eysker and Ploeger, 2000). The correlation between serum pepsinogen concentration and GIN burden is reduced once animals are housed (as there is no new exposure to infective larvae), or in mature cattle. In fact, elevated blood pepsinogen values can also be found in clinically healthy cows (Berghen et al., 1993), probably as a result of hypersensitivity following previous burdens (Mylrea and Hotson, 1969). This implies that for the diagnosis of ostertagiasis, pepsinogen values always have to be used in conjunction with clinical and parasitological data, making them a labour-intensive method of diagnosis.

1.5.2.2 Determination of Ostertagia ostertagi antibody concentration

A diagnostic alternative to the FEC is the use of immunological determination of antibody titers against GIN through enzyme-linked immunosorbent assay (ELISA) (Eysker and Ploeger, 2000).

Recently, a commercial assay for quantifying antibodies against *Ostertagia ostertagi* in cattle has been made available (SVANOVIR® *Ostertagia ostertagi*-Ab ELISA). The SVANOVIR® *Ostertagia ostertagi*-Ab ELISA assay is designed for use in adult dairy cows using milk samples (Charlier et al., 2009). In the dairy industry, a negative relationship has been established between bulk tank milk antibody level and measures of milk production, reproduction and mortality (Delafosse, 2013; Forbes et al., 2008; Sanchez et al., 2005; Vanderstichel et al., 2012). Sanchez et al. (2002; 2005) in studies in Canadian dairy herds found that an Optical Density Ratio (ODR) greater than 0.4 to 0.5 obtained with the ELISA was related to a positive response in milk-production following anthelmintic treatment. Assessment of antibody titers has not been investigated as a potential diagnostic tool for GIN in cow-calf herds, in part because the economic impact of subclinical GIN burdens have to date not been well elucidated in this industry (Colwell et al., 2014).

The use of an ELISA to evaluate antibody levels to quantify GIN burden raises concerns for its use in adult cattle because of the persistence of antibodies in more mature cattle. Adult cattle may, demonstrate ODR that overestimate their current exposure levels to *O. ostertagi*. Eysker & Ploeger (2000) also suggest that because of the widely varying ability to develop an immune response against GIN, the antibody ELISA may be best used for determining herd level exposure as opposed to quantifying GIN burden at the individual cow level.

1.6 Determination of larval species identity

The accurate identification of parasites and the proportional composition of mixed burdens - regardless of the developmental stage - have important implications for helping to understand the epidemiology, diagnosis, treatment and control of GIN in grazing ruminants (Avramenko et al., 2015; Roeber et al., 2013).

Parasites are frequently identified by examination of morphology, the host they infect, their transmission patterns, their pathological effects and the location where they are found. The morphological similarities of GIN eggs and larvae mean this method is often insufficient for specific identification of the GIN found in burdens in beef cattle. Given that GIN genera and species vary in their pathogenicity, epidemiology, the accurate diagnosis and quantification of the different species that are involved in GIN burdens in cattle is essential to sustainable control (Avramenko et al., 2015; Roeber et al., 2013).

1.6.1 Larval culture and larval differentiation

Coproculture and differentiation of L3 larvae microscopically based on morphology have historically been used. The samples cultured may be from an individual or a group. These methods while widely used have significant limitations in the quantitative determination of larval species identity of GIN of importance to beef cow-calf production. Some of the factors that can affect the use of larval culture and morphological identification of GIN include:

1. Loss of viability of eggs in transit from property to lab
2. Competition between species during larval culture
3. Differential sensitivity of species to development at culture temperature
4. Differential development time between species
5. Low throughput and time consuming
6. Prone to subjectivity and human error

The difficulties and limitations in current larval differentiation techniques described above, new methods are being explored including advanced molecular technologies (Avramenko et al., 2015; Roeber and Kahn, 2014; van Wyk et al., 2004)

1.6.2 Molecular technology

Molecular technologies are emerging as a possible methods for solving some of the diagnostic issues encountered when working with GIN of cattle (Gasser, 2006). Traditionally, FEC followed by fecal culture with larval morphological analysis or polymerase chain reaction (PCR) are used to confirm species in GIN burdens (Charlier et al., 2014).

An emerging technique in parasitological research used to accurately quantify the species composition of cattle GIN burdens is a deep sequencing assay of the internal transcribed spacer 2 (ITS-2) region of the nematodes' rDNA. This method was described and validated by Avramenko et al. (2015). The ITS-2 region of the rDNA contains species-specific variation which allows for reliable identification of GIN species (Avramenko et al., 2015). The results of this study show that the relative quantity of the different GIN species that make up mixed

burdens in cattle could be accurately described. Advantages of this new technique over conventional larval culture include the accurate differentiation of species that are morphologically identical and - compared to the FEC followed by larval culture - it enables a high throughput. One of the disadvantages of this method is it still does not allow for the quantification of intensity of GIN burden.

1.7 CANADIAN BEEF INDUSTRY

Canada in 2014 produced 1.41 million tonnes of beef making it the 5th largest exporter of beef products globally (Canfax Research Services, 2015; Statistics Canada, 2015). This makes it a very important sector of the Canadian agrarian economy, contributing up to $33 billion CAD annually (Kulshreshtha et al., 2012). The beef industry in Canada can be described broadly as having three sectors; 1) Cow-calf herds that breed cows to produce calves; 2) Stocker or backgrounding herds where producers take weaned calves from cow-calf herds and place them on pasture or feed them forage-based diets to add body weight, and 3) Feedlot operations that accept either weaned calves from cow-calf herds or stocker calves from backgrounding operation and feed them grain-based diets until they reach slaughter weight.

While a wide range of production systems are seen, from animal numbers to production management programs utilized in the Canadian beef cow-calf sector, there are some basic similarities across the country (Sheppard et al., 2015). Starting with the breeding of replacement heifers as yearlings that then calve at approximately 2 years of age (Mathison, 1993). Across the major beef producing areas of Canada, most calving occurs in the winter–spring (January to May) (Mathison, 1993; Sheppard et al., 2015). Calves are then weaned in the fall (October or November) at an average of seven months old of age. While winter confinement and feeding is common, extended grazing (e.g. swath, stockpiled and bale grazing) is seen in the beef cow-calf sector (Sheppard et al., 2015). Beef cow-calf pairs are then grazed on pasture from spring/summer to early fall.

In the 2016 Canadian Agricultural Census 61,425 farms in Canada reported having beef cows and calves this is a 9.5% decrease from the last Agricultural Census in 2011. However, in the same time period the average number of beef cows on these farms have increased 12.5%, resulting in larger herds. In 2016 65% (35,189 farms) were in the three western Canadian prairie provinces of AB, SK and MB. Of those, 48.4% were in AB, 35.3% in SK and 16.3% in MB

(Statistics Canada, 2016). A similar trend is seen when looking at the total number of beef cows. In 2016 3,732,555 beef cows were reported in Canada. Of these 83% were reported in the three prairie provinces. Making this region and its beef cow-calf production systems the focus of this thesis.

1.8 Gastrointestinal nematode management in cow-calf herds

Eradication of infective GIN larvae on pasture is unlikely even with the use of the strictest management strategies (Myers and Taylor, 1989; Scott and Sutherland, 2009). When designing programs then to manage GIN burdens we must consider the main sources of infection (Waller, 2006). For GIN in grazing beef cattle this is the free-living stage of the parasite on pasture and the parasites that live within the animal. The degree to which pastured cattle are parasitized by GIN is determined by many factors including, physiological state of the animals, predominant nematode species and seasonal conditions. A lot of these factors are beyond beef cow-calf operator's control. Therefore the management of GIN by beef cow-calf operators is based on factors that they can employ to limit animal exposure to GIN on pasture and anthelmintic drug administration (Stromberg and Averbeck, 1999; Waller, 2006).

1.8.1 Grazing management

The type of grazing system used in a beef operation can play a part in the number of infective larvae on pasture. Michel (1985) suggested that there are three overarching grazing management strategies that can be utilized to control animal exposure to infective L3 larvae on pasture. These methods include:

1. Preventive- for example the grazing of pasture with different species of livestock, which results in a reduction in infective L3 larvae that do not infect both species (Sutherland & Scott, 2010). Methods of reducing the intake of infective larvae (L3) also include the provision of 'clean' pasture, for example newly sown or pastures rested for a long period of time (Stromberg & Averbeck, 1999; Sutherland & Scott, 2010).
2. Evasive- these grazing management strategies include rotational systems. Rotational grazing involves the planned movement of animals from one pasture to another, resulting

in a period of time, which each pasture is ungrazed. The effectiveness of these methods relies on many factors, including stocking density/time in each pasture, rate of pasture regeneration and age/physiological state of the animals on the pasture. There is some evidence that rotational grazing results in increased infective L3 larvae on pasture and because of this increased infection pressure on animals, because of increased stocking densities and thus closer grazing to fecal pats. (Stromberg and Gasbarre, 2006).

3. Dilutive- the idea behind this method of grazing management is to graze older and so more immunocompetent animals with susceptible young stock or different livestock species to dilute pasture larval infestation resulting from their combined fecal output of parasite eggs. Craig (1988) found that the majority of eggs are shed by the younger animals. However, cows act as reservoirs for parasites that shed infectious eggs. This results in increased pasture contamination, by passing the egg burden in cows onto young stock.

1.8.2 'INTEGRATED', 'STRATEGIC' AND 'TARGETED SELECTED' GASTROINTESTINAL NEMATODE CONTROL

There is agreement that when considering GIN management 'integrated' and 'strategic' management programs will need to be utilized in order to maximize animal health/welfare, animal production and to slow the development of AR to the anthelmintic drugs available to beef cow-calf producers. The 'integrated' management of GIN involves the considered use of both grazing management and anthelmintic drug administration and 'strategic' management relies the responsible use of anthelmintic drugs in order to protect their effectiveness. The success of these integrated and strategic management programs relies on utilizing the most current epidemiological information about the GIN species present in specific locations, local climatic conditions, and age of the animals and herds management practices. These programs also rely on the responsible use of anthelmintic drugs by beef cow-calf operators, including appropriate administration methods and calculation of treatment dose (Kenyon and Jackson, 2012).

The overarching aims of both 'integrated' and 'strategic' management programs are to limit the contamination of pasture with infective L3 larvae, limit animal exposure to the infective L3 larvae and reduce the burden of GIN within animals. In order for programs to do this they rely significantly 'on knowledge of the relationship between the parasites and their host' and consideration of local environmental conditions (Stromberg and Gasbarre, 2006). There has been

little to no work done recently to establish the effect that differences in management strategies and GIN species composition have on subclinical GIN burdens in western Canadian beef cow-calf herds. It is important that these knowledge gaps be addressed in order for better 'integrated' and 'strategic' management programs to be developed for these herds.

Unfortunately, the current management programs utilized by beef cow-calf herds in Canada most often rely on the routine and repeated blanket treatment of animals, both young and mature cattle, at times that are convenient in a herd's management schedule, rather than integrating treatment with the epidemiology of the GIN species present and considering local climatic conditions. The use of topical macrocyclic lactones to treat ectoparasites, a lack of available knowledge, as well as labor and infrastructure considerations that result in the overuse of anthelmintic drugs in GIN management programs in western Canadian cow-calf herds. This overuse of anthelmintics raises the concern of advancing the development of AR (Mackie, 2016).

In order to reduce the development of AR, not only does the responsible use of anthelmintic drugs need to be considered in respect to GIN epidemiology and climate but also the number of animals that are being treated. The idea of 'refugia' has been highlighted as a concept that is important in reducing the development of AR. Refugia is basically the idea of having a pool of 'untreated' or 'susceptible' GIN on pasture which dilute resistant nematodes surviving anthelmintic treatment and, hence, reducing selection pressure for the development of AR (Greer et al., 2009; Jackson et al., 2006; Waghorn et al., 2009). However, managing GIN while maintaining a 'refugia' population may increase the risk of parasitism and production losses. Refugia-based approaches can include either changes to the timing and/or the frequency of anthelmintic treatments, as has been discussed above.

The second way of maintaining a refugia population is to utilize more 'targeted selective' treatment strategies (TST), where only those animals which the highest burden of GIN in a herd are treated (Besier, 2012). The difficulty in utilizing TST in beef cow-calf herds is being able to identify those animals with the heaviest GIN burdens in order to treat them. In cattle, traditional methods used to identify animals in need of treatment, such as production parameters (e.g. BCS) or diagnostic measures (e.g. FEC) do not correlate well with actual GIN burden (Eysker and Ploeger, 2000; Nodtvedt et al., 2002). Therefore, alternative diagnostic techniques need to be investigated, such as the anti-*Ostertagia ostertagi* antibody ELISA discussed above, before TST can realistic be utilized by beef cow-calf herds.

1.8.3 ANTHELMINTICS

The effective treatment of GIN was revolutionized in the 1960's with the development of new anthelmintics. This progress in the treatment of GIN continued into the 1980's with the continued production of new, safe and effective anthelmintic classes (McKellar and Jackson, 2004). In Canada, two major classes of broad-spectrum anthelmintic drugs are used in the control of GIN in cattle. Perhaps the most widely used are the macrocyclic lactones (including ivermectin, doramectin, moxidectin and eprinomectin). The second class of commonly used anthelmintics is the benzimidazoles, which includes albendazole and fenbendazole. There is also a third class of anthelmintic drug registered, the imidazothiazoles, with levamisole being the most commonly used.

Effective control of GIN in cattle with anthelmintics has positive impacts on productivity, weight gain, feed conversion, milk production, reproductive performance, carcass quality, immune status and morbidity and mortality (Stromberg et al. 1997; Bauck et al. 1989). This led to the development of treatment programs that focused on the routine blanket treatment of livestock with anthelmintics (Charlier et al., 2014). The frequent use of many anthelmintics that are used for the treatment of GIN in beef cattle – particularly macrocyclic lactones – has been compounded by the fact that they are also used for the treatment of ectoparasites. The availability of effective, safe and inexpensive anthelmintics has allowed for the use of GIN management programs that rely solely on anthelmintic drugs, as opposed to alternate control practices - such as pasture rotation - even under the more intensive stocking practices utilized today (Stromberg and Gasbarre, 2006).

While the routine blanket treatment of livestock with anthelmintics for the control of GIN burdens has been profitable for cattle operators, this practice also places increased selection pressure on GIN populations leading to the development of anthelmintic resistance (AR). This has been well documented in small ruminants worldwide (Stromberg and Gasbarre, 2006) and as Sunderland and Leathwick (Sutherland and Leathwick, 2011) point out: "It would seem obvious that no country or industry should consider themselves immune from the threat of anthelmintic resistance".

1.8.3.1 ANTHELMINTIC RESISTANCE

The detection of AR in cattle parasite populations is more challenging than in small ruminants. This is largely in part because of the fact that FEC are generally lower in cattle - particularly adult cattle - than in sheep or goats, making it more difficult to detect the reduction in FEC post treatment and thus calculate anthelmintic treatment efficacy (Sutherland and Leathwick, 2011).

Anthelmintic resistance can be diagnosed using FEC to determine the portion of L4 and/or adults that are able to survive treatment. This test is referred to as a fecal egg count reduction test (FECRT). The World Association for the Advancement of Veterinary Parasitology (WAAVP) published methods for the detection of AR in nematodes of veterinary importance. The WAAVP guidelines state that, in order for no AR to be present, there should be a reduction in GIN FEC of at least 95% following treatment, or a lower confidence interval of the FEC of >90% (Coles et al., 1992). More recently, the guidelines for FECRT in cattle have been reviewed by Levecke et al. (2012a) and Coles et al. (2006), to help address some of the difficulties of conducting FECRT on cattle, including using larger sample sizes, higher mean pre-treatment FEC and low test detection limits.

The development of resistance varies depending on GIN species, anthelmintic class and geographical location. Reports of macrocyclic lactone resistance in beef cattle have been made for *Ostertagia ostertagi, Cooperia* spp. as well as *Haemonchus* spp. from several continents including North America, Europe and Australia (Anziani et al., 2004; Areskog et al., 2013; Edmonds et al., 2010; Gasbarre et al., 2009b). A study including 72 cow-calf herds found that treatment with an oral benzimidazole and injectable or topical macrocyclic lactone failed to reduce FEC by >90% in 1/3 of the herds (USDA, 2010). All herds that achieved less than a <90% reduction had used a pour-on macrocyclic lactone as the anthelmintic treatment (USDA, 2010). Benzimidazole resistance in beef cattle has also been reported in multiple countries (Chaudhry et al., 2014; Gasbarre et al., 2009a, 2009b; Mejia et al., 2003).

At this time, there are no published studies examining the development of AR of anthelmintic products in cattle in western Canada. Although data evaluating the effectiveness of both fenbendazole and ivermectin in beef cows and calves from one operation in Ontario suggested evidence of resistance or reduced efficacy to both drugs (Mackie, 2016).

1.9 Conclusions

It has been effectively shown that there is a cost to beef cow-calf herds from GIN burdens and that through the management and treatment of these burdens, there is an economic benefit to producers. Despite this there has been little current research done in western Canada to quantify the prevalence, fecal intensity and species composition of GIN on beef cow-calf herds, that is not specific to a specific operation, animal production type, treatment or time period. Continuing research should be focused on defining the current impact of GIN burdens in western Canadian cow-calf herds and in particular address techniques that could be used quantitatively diagnose both FEC intensity and species composition so that perhaps a more accurate estimate of the economic impacts of these burdens can be established for these operators.

Beyond this there is also a lack of current research examining the epidemiology of GIN in the cooler temperate climates of the western Canadian prairie provinces. Because of the large effect that environment has on the nematodes' lifecycle, in particular larval development and survivability, local knowledge must be derived for the beef sector to allow it to develop responsible and sustainable management strategies for these parasites. Future research needs to be conducted firstly into defining the current management strategies utilized by beef cow-calf operators of western Canada and also defining the current epidemiology of these parasites in the 'local' environment, so that management strategies can be can be developed and tailored specifically to the 'local' environment and herd management practices.

1.10 Hypothesis and objectives

Gastrointestinal nematodes (GIN) are a threat to the health and welfare of cattle worldwide and their substantial detrimental effects on productivity in beef cattle are well documented (Forbes et al., 2002). Even low levels of GIN have negative effects in beef cattle, as demonstrated by Kunkle (Kunkle et al., 2013). Young cattle on pasture (such as calves on cow-calf herds and yearlings) are most at risk of acquiring high levels of parasites because of a naïve immune system, with adult cows acting as the source of pasture contamination and infection. Preventive approaches to decrease parasite burden must be utilized on cow-calf herds. There is a paucity of current information available on the level of parasitism in grazing beef cattle in western Canada.

Several changing risk factors have been identified that indicate we should investigate current prevalence and intensity of GIN in beef cattle in western Canada. Locally changes in beef

cattle management practices such as increased herd sizes later spring calving, and altered feeding and grazing practices may have potentially increased infection pressures (Jelinski et al., 2015; Waldner et al., 2013). Globally the epidemiology and species of GIN may be effected by changing climates (Fox et al., 2015; Gethings et al., 2015). Also evidence of emerging AR documented in beef cattle globally (Sutherland and Leathwick, 2011), necessitate we understand the level of parasitism that exists on cow-calf herds in western Canada today, both with regards to fecal egg shedding intensity and predominant species. Risk factors including current anthelmintic treatment protocols and pasture management need to be characterized to develop economically sound and sustainable control practices to decrease the impact of internal parasites on production and limit the continuing development of AR.

In summary, the aim of this thesis is to address gaps in current knowledge about the epidemiology and management of gastrointestinal nematodes on cow-calf herds in western Canada. Addressing these knowledge gaps will help direct development of evidence based, sustainable parasite control programs that maintain current beef production levels while decreasing the risk of the development of AR. This will make the western Canadian beef industry more resilient in the long-term and aid in continued economic production of quality meat.

Therefore, the objectives of this thesis are:

1. To determine the prevalence and fecal egg count intensity of gastrointestinal nematode burdens in different production types (cows, calves and replacement heifers) from cow-calf herds of the western Canadian prairie provinces.
2. To characterize the herd-level gastrointestinal nematode burden of western Canadian cow-calf herds quantitatively and qualitatively by conducting fecal egg counts and determination of larval species identity.
3. To characterize the current management strategies employed by western Canadian cow-calf producers in the control of gastrointestinal nematodes, from the responses to a questionnaire administered to these producers.

CHAPTER TWO

GASTROINTESTINAL NEMATODE PREVALENCE AND FECAL EGG COUNT INTENSITY IN BEEF CATTLE FROM WESTERN CANADA

Felicity K. Wills[1]; Colleen Pollock[2]; John R. Campbell[1]; Cheryl L. Waldner[1]; Fabienne D. Uehlinger[1].

[1]From the Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK, S7N 5B4, Canada.
[2]From Merck Animal Health Canada, Intervet Canada Corp., 10055 106 Street, Suite 600, Edmonton, AB T5J 2Y2

This chapter contains the analysis of data provided by Merck Animal Health Canada of fecal egg counts collected from beef cow-calf herds from the western Canadian prairie provinces. This chapter represents the first description of the prevalence and FEC intensity of gastrointestinal nematode burden in beef cattle from the western Canada, that spans the entire geographical area of the prairie provinces and which looks a multiple production types. There has not been a large scale description of gastrointestinal nematode burdens in western Canada since Bickis and Polley (1987)*, and with gastrointestinal nematode burdens, even at subclinical levels, causing production loses it is important to have a current description of FEC burden prevalence and FEC intensity, in order for management decisions to be made. The results of this chapter revealed a consistently high prevalence but low-level FEC intensity of gastrointestinal nematodes in these herds, across all animal production types. The high prevalence of gastrointestinal nematode burdens indicates the potential for substantial production impacts to be effecting beef cow-calf herds of western Canada. Further research into the current epidemiology,*

determination of larval species identity, and management of gastrointestinal nematode burdens on beef cow-calf herds of the western Canadian prairie provinces, is warranted. It will be the aim of the remaining chapters of this thesis to describe the epidemiology, species composition and management of gastrointestinal nematodes in these herds.

AUTHOR CONTRIBUTIONS: Pollock was responsible for the collection of samples and delivery of the data set. Uehlinger, Campbell and Waldner were responsible for design of the data analysis and manuscript review. Wills was responsible for design and analysis of data and manuscript preparation.

CONTRIBUTIONS: Fecal sample laboratory processing was performed by BioCheck Veterinary Diagnostics and Technologies. Lethbridge, AB, Canada.

2.1 Abstract

Gastrointestinal nematodes (GIN) are a threat to the sustainability of livestock production worldwide through productivity loss and treatment costs. Understanding the epidemiology of gastrointestinal nematodes in a specific region is critical in developing efficient and effective control programs. There is a paucity of current information on the prevalence and FEC intensity of GIN burdens in beef cow-calf herds from western Canada. In this study, fecal samples from cows, calves and replacement heifers (n=3,891) collected by Merck Animal Health Canada during 2012, 2013 and 2014 from 240 herds were examined. A saturate sugar and centrifugation technique was used to perform individual fecal egg counts. Trichostrongylid-type eggs, *Nematodirus* spp. and *Trichuris* spp. were differentiated morphologically microscopically. Replacement heifers had the highest predicted prevalence of Trichostrongylid-type eggs at 81% (95% CI 72-90), and cows had the lowest prevalence at 75% (95% CI 72-80). Calves had the highest predicted mean egg count of Trichostrongylid-type eggs overall at 5.8 (95% CI 3.5-8.3), while heifers had the lowest predicted mean EPG at 3.9 (95% CI 2.9-4.9). *Nematodirus* spp. egg positive samples were seen most frequently from calves, with a predicted prevalence of 36% (95% CI 30-42). *Trichuris* spp. eggs were very infrequent at an overall raw prevalence of 0.2% (7/3891; 95% CI 0.08 – 0.4). The high prevalence of GIN - even with a low FEC intensity - with their potential to cause significant production losses, highlights the need for further investigation of the epidemiology of gastrointestinal nematodes in western Canada. This is particularly relevant considering increasing herd sizes, management changes, climate change and the development of anthelmintic resistance that have all been identified as potential factors that may influence the impact of GIN on beef cow-calf herds in western Canada.

2.2 Introduction

Gastrointestinal nematodes (GIN) are a threat to the sustainability of livestock production worldwide through productivity loss and treatment costs (Morgan et al., 2013; Vercruysse and Claerebout, 2001). While parasitic gastroenteritis (PGE), characterized by diarrhea, anorexia and weight loss, primarily affects young cattle during their first grazing season, overt clinical disease is now rarely seen in North America; this is largely because of the regular use of effective anthelmintic products over the last decades (McArthur and Reinemeyer, 2014). However, the detrimental effects of subclinical GIN parasitism on productivity in grazing beef cattle include poor growth rates, reduced reproductive efficiency and reduced calf weaning weights (Forbes et al., 2002; Hawkins, 1993; Loyacano et al., 2002).

Gastrointestinal nematode control programs in cattle have relied on intensive 'blanket' anthelmintic treatments aimed at preventing the accumulation of parasite burdens (McArthur and Reinemeyer, 2014). This approach has been based on observed increases in animal productivity following the application of effective anthelmintic drugs (MacGregor et al., 2001; Reinhardt et al., 2006). Research suggests that the routine blanket treatment of beef cattle with anthelmintic drugs has led to increasing anthelmintic resistance (AR) (Gasbarre, 2014; Vercruysse and Claerebout, 2001). Anthelmintic resistance in nematodes of cattle have been reported in the United States, New Zealand, Australia, Argentina, Brazil, and Europe against all classes of anthelmintic products licensed for use in beef cattle in Canada (Cotter et al., 2015; Gasbarre, 2014; Kaplan and Vidyashankar, 2012; Sutherland and Leathwick, 2011).

Gastrointestinal nematode burdens vary with geographic location. This variation can partly be because of regional differences in animal and pasture management and also because of species specific differences between the survivability of infective larvae in different environments (Wilson et al., 2001). Understanding the epidemiology of GIN in a specific region is critical in developing efficient and effective control programs (Waller, 2006). Beef cow-calf herds in western Canada represent an economically important sector of the Canadian agrarian economy (Kulshreshtha et al., 2012). This sector has changed significantly over the last decade. The number of herds reporting cow-calf production has reduced while the size of herds has increased (Beaulieu, 2015; Statistics Canada, 2014). Additionally, calving is occurring later in the spring (Jelinski et al., 2016). Altered grazing and changing climates have also been identified as potential risk factors for increasing GIN burdens (Fox et al., 2015; Gethings et al., 2015;

Stromberg and Averbeck, 1999; Yazwinski and Tucker, 2006).

There is very limited information on the epidemiology of GIN in beef cow-calf production systems in western Canada. Given observed changes in climate and herd management, a better understanding of the epidemiology of GIN in beef cow-calf herds in this region is needed to develop strategic control programs which optimize production while limiting the risk of increasing development of AR. The objective of this study was to describe the prevalence and FEC intensity of GIN burdens in different animal production types of beef cow-calf herds in western Canada between 2012 and 2014.

2.3 MATERIALS AND METHODS

2.3.1 STUDY POPULATION AND SAMPLING

During 2012, 2013, and 2014, samples were collected from cows, calves, and replacement heifers from cow-calf herds in western Canada. Fresh environmental fecal samples were collected from individual animals after observed defecation. The sample population consisted of a convenience sample of beef producers visited by a Merck Animal Health (Canada) representatives or by the farm's regular herd veterinarian. There was no repeat sampling of the same properties or cattle over successive seasons or years. Date of collection, the date of last treatment with an anthelmintic, product name, and the production type of the animal sampled (cow, calf or replacement heifer) were recorded where possible at time of collection. Fecal samples were individually sealed in plastic bags and shipped to the laboratory (BioCheck Veterinary Diagnostics and Technologies. Lethbridge, AB, Canada) on ice within 24h of collection.

2.3.2 LABORATORY ANALYSIS

Individual fecal samples were processed using a saturated sugar flotation and centrifugation technique according to the laboratory's (BioCheck Veterinary Diagnostics and Technologies. Lethbridge, AB, Canada) protocol. In brief, 3g of feces were mixed with 15ml of a saturated sugar solution (specific gravity 1.27) to create a fecal slurry. This slurry was strained through a course sieve and placed into a test tube, which was centrifuged (relative centrifugal force (RCF) 180 x g) at 900 rotations per minute (rpm) for 7 minutes. The test tube was then

placed on a flat surface and filled to a slight convex meniscus with the saturated sugar solution and a cover slip placed on top. The samples were then left to stand for at least 30 minutes. The cover slip was removed and placed on a microscope slide for examination at 40x magnification. These flotation and centrifugation methods have been shown by Egwang and Slocombe (1981) to detect samples with egg fewer than seven eggs per gram. Gastrointestinal nematode eggs were identified microscopically based on morphology as Trichostrongylid-type, *Nematodirus* spp. or *Trichuris* spp. and reported as eggs per three grams of feces (EP3G). Eggs per gram of feces (EPG) was calculated by dividing the egg counts by the original weight of the sample.

2.3.3 Data analyses

The information collected at time of sampling and the fecal egg counts were entered into a commercial spreadsheet program (Excel 2013; Microsoft Corp., Redmond, Washington, USA) for data checking and then imported into a statistical software package (StataSE version 14, Stata, College Station, Texas, USA) for analysis. Based on the collection date, samples were categorized into season of collection: either the spring/ summer grazing period (March, April, May, June, July and August) or fall (September, October and November). Because very few samples were collected in winter (December, January, and February), they were omitted from the analyses. Samples were further classified by production type (cows, calves and replacement heifers). Submissions that were known to have been treated with macrocyclic lactones within 45 days or with benzimidazoles within 15 days before sample collection were also excluded from further analyses (Taylor and Hodge, 2014).

Fecal samples were described for each year, season and production type. Raw descriptive statistics included the prevalence (exact binomial 95% confidence interval (CI)) and the geometric mean (SD), range, and median (IQR) EPG of Trichostrongylid-type, *Nematodirus* spp. and *Trichuris* spp. positive samples (Appendix Tables A2.1 to A2.6). No *Trichuris* spp. were found in heifers in any of the years and *Trichuris* spp. was also not identified in any of the sampled cattle in 2013. The overall low prevalence of *Trichuris* spp. in samples meant, subsequent analyses were restricted to Trichostrongylid-type eggs and *Nematodirus* spp. only.

The overall prevalence (95% CI) of Trichostrongylid-type eggs was estimated using generalized estimating equations (GEE) to allow for clustering within herds. The initial null or

intercept only GEE model used a binomial distribution and logit link function with an exchangeable within-group correlation structure and robust standard error (to deal with overdispersion within the data). The overall mean Trichostrongylid-type EPG were also determined using a null GEE model with a negative binomial family and log link with an exchangeable within-group correlation structure and robust standard error. The prevalence (95% CI) of *Nematodirus* spp. positive samples and mean EPG for *Nematodirus* spp. were estimated in the same way as for Trichostrongylid-type described above.

The effects of year, season and production type on the Trichostrongylid-type prevalence and EPG burdens in cows, calves and heifers were also assessed with fixed effects introduced in the above GEE models. Each independent variable (year, season and production type) was forced into the final model and plausible interaction terms (year by season, year by production type, season by production type and year by season by production type) were evaluated. The final GEE model for each outcome was produced by manual stepwise backwards elimination of non-significant interaction terms. Interaction terms were retained in the GEE if found to be statistically significant based on a Wald's test at a p-value of ≤ 0.05. The effect of retained predictor variables on the predicted prevalence and EPG of Trichostrongylid-type positive samples were assessed using post-hoc pairwise comparison with a level of significance set at p-value ≤ 0.05. Similar analyses were completed to estimate differences in *Nematodirus* spp. prevalence and mean EPG among years, seasons and production types.

2.4 RESULTS

2.4.1 SAMPLE POPULATION AND NUMBER OF SAMPLES COLLECTED

From 2012 to 2014, 3,891 fecal samples suitable for analyses were collected from 201 herds. Table 2.1 shows the number of cows, calves and replacement heifers sampled by year and season of sample collection. The number of samples collected from each herd ranged from 4 to 57 samples (median 20, IQR 6). The greatest numbers of samples were collected in the summer months of each year (75.5% (1514/2004) in 2012, 67.1% (1001/1492) in 2013, and 58.7% (382/651) in 2014). In each year, the most samples were collected from cows (45.1% (904/2004) in 2012; 45.6% (680/1492) in 2013; 57.4% (419/730) in 2014).

2.4.2 Raw and unadjusted prevalence and FEC intensity of Trichostrongylid-type eggs, Nematodirus spp. and Trichuris spp.

Raw descriptive statistics for prevalence and mean EPG burden of Trichostrongylid-type eggs, *Nematodirus* spp. and *Trichuris* spp. are depicted in the Appendix (Tables A2.1 to A2.6). There were seven *Trichuris* spp. positive samples identified from 4 herds. Three positive samples came from calves from one herd. A second herd had 2 cows sample positive and the third herd, had one cow sample positive. One final herd from had a single positive calf. The low frequency and FEC intensity of *Trichuris* spp. resulted in no further analysis being conducted.

The predicted prevalence and mean EPG of Trichostrongylid-type egg positive samples from the null models were 78% (95% CI 75-82; Table 2.2) and 4.9 (95% CI 3.9 – 5.9; Table 2.3) EPG, respectively. For *Nematodirus* spp., the null-model derived predicted prevalence was 14% (95% CI 11-17; Table 2.4) while the predicted mean EPG of *Nematodirus* spp. eggs was 0.4 (95% CI 0.3 – 0.6; Table 2.5).

2.4.3 Final GEE for the predicted prevalence of Trichostrongylid-type eggs in fecal samples

After the manual stepwise backward elimination of non-significant interaction terms, the final GEE model for the predicted prevalence of Trichostrongylid-type egg positive samples included year (p= 0.005) and season (p=0.0009) and a significant interaction between season and production type (p=0.01) (Table A2.7). Production type (p=0.70) was not statistically significant alone in this model.

Table 2.2 shows the predicted prevalence of Trichostrongylid-type egg positive samples from the final GEE. The predicted prevalence of Trichostrongylid-type egg positive samples differed significantly between years, with 2012 having a significantly (p=0.001) higher predicted prevalence (84; 95% CI 79-88) than 2013 (71; 95% CI 65-77). Overall, the predicted prevalence in the cows was significantly lower compared to calves (p=0.03) and heifers (p=0.028); there was no significant difference between calf and heifer prevalence (p=0.86). There was also no significant difference in the overall predicted prevalence between seasons (p=0.33). There was, however, a significant interaction between season and production type. While the predicted prevalence of *Trichostongylid*-type egg positive samples increased (but not significantly) in both

calves and replacement heifers from spring/summer to the fall, the predicted prevalence in cows fell significantly (p=0.001) between those seasons from 81 to 55%. Cows sampled in the fall had the lowest predicted prevalence at 55% (95% CI 39-71). The predicted prevalence of cows in the fall was significantly lower than the prevalence in both calves and heifers in spring/summer (p=0.008 and p=0.005, respectively) and the fall (p=0.006 and p=0.005, respectively).

2.4.4 Final GEE for the predicted mean Trichostrongylid-type eggs per gram of feces

After the manual stepwise backward elimination of non-significant interaction terms, the final GEE model for the predicted mean EPG of Trichostrongylid-type eggs included year (p=0.13) and season (p<0.001), and a significant interaction between season and production type (p<0.001) and between year and production type (p=0.001) (Table A2.8). Production type (p=0.14) was not significant alone in this model.

Table 2.3 shows the predicted mean EPG count of Trichostrongylid-type eggs from the final GEE. There was no statistically significant difference in the overall mean Trichostrongylid-type EPG between seasons (p=0.11). However, the predicted mean in 2012 (5.8; 95%CI 4.2-7.3) was significantly (p<0.001) higher than in 2013 (3.0; 95%CI 2.5-3.7) (Table 2.3). Calves had a significantly higher predicted mean EPG compared to cows (p<0.001) but there was no difference between calves and heifers (p=0.15) or heifers and cows (p=0.22).

When the significant interaction between production type and season was examined, cows sampled in the fall had the lowest predicted mean Trichostrongylid-type egg count at 1.6 (95% CI 1.0-2.2). Cows' predicted mean EPG in the fall differed significantly from their mean EPG in spring (p<0.001) as well as from that of calves and heifers in spring (p<0.001 and p=0.002, respectively) and fall (p<0.001 and p=0.021, respectively; Table 2.3). The predicted mean EPG of heifers in the spring/summer differed significantly from that of cows in the spring/summer (p=0.043) and of calves in the fall (p=0.041).

There was a significant interaction between year of sample collection and production type. For example, the overall predicted mean EPG in calves in 2014 was significantly higher than that in cows (p<0.001) and heifers (p=0.012; data not shown). Similarly, the predicted mean EPG in heifers in 2013 was significantly higher compared to that in cows (p=0.009) and calves (p=0.021; data not shown). Furthermore, the predicted mean EPG in calves differed between

years and was significantly higher in 2012 and in 2014 compared to 2013 ($p<0.001$; data not shown).

2.4.5 Final GEE for the predicted prevalence of Nematodirus spp.

The final GEE model for the predicted prevalence of *Nematodirus* spp. included year ($p<0.01$), season ($p=0.02$) and production type ($p<0.01$) (Table A2.9). None of the tested interaction terms were significant. Table 2.4 shows the predicted prevalence of *Nematodirus* spp. egg positive samples from the final GEE.

The predicted prevalence was significantly higher in 2012 (20%; 95% CI 16-25) than in 2013 (8%; 95% CI 5-10; $p<0.001$) or 2014 (11%; 95% CI 5-17; $p=0.04$). There were also significantly more ($p=0.02$) *Nematodirus* spp. positive samples in the fall (21%; 95% CI 13-29) than in the spring/summer (12%; 95% CI 10-15). Calves had the highest predicted prevalence of *Nematodirus* spp. at 36% (95% CI 30-42), which was significantly higher than the predicted prevalence for cows (3%; 95% CI 1-6; $p<0.001$) and replacement heifers (6%; 95% CI 2-10; $p<0.001$).

2.4.6 Final GEE for the predicted mean Nematodirus spp. eggs per gram of feces

The final GEE model for the predicted mean EPG count of *Nematodirus* spp. included year ($p<0.001$), production type ($p<0.001$) and season ($p<0.001$) (Table A2.10). There was no significant interaction between year and production type ($p=0.09$), year and season ($p=0.4$) or season and production type ($p=0.46$). Table 2.5 shows the predicted mean EPG count of *Nematodirus* spp. eggs from the final GEE model.

The predicted mean *Nematodirus* spp. EPG was significantly higher in 2012 compared to 2013 ($p<0.001$) and 2014 ($p=0.001$). Calves had the highest predicted mean *Nematodirus* spp. EPG at 1.2 (95% CI 0.7-1.6) which was significantly higher than the predicted mean EPG for cows and heifers (both p-values <0.001). The predicted mean *Nematodirus* spp. EPG was also significantly higher in the fall than in the summer ($p<0.001$).

2.5 Discussion

There are few studies available in western Canada that report the prevalence or FEC intensity of GIN burdens in beef cattle. Beef cow-calf production in western Canada encompasses over 70% of all cow-calf beef production. This study represents a wide cross-section of cow-calf herds representing the entirety of the prairie provinces, allowing for a more representative sampling of an important sector of the Canadian agrarian economy (Statistics Canada, 2017).

Trichostrongylid-type egg prevalence was high with 78% of all samples positive. The prevalence of *Nematodirus* spp. and *Trichuris* spp. was lower, with *Trichuris* spp. being a very infrequent finding at 0.2% of all samples. This pattern in the prevalence of the morphologically identifiable types of Trichostrongylid-type eggs is similar to that described by Jelinski et al. (2016), who sampled 14 beef cow-calf herds over summer 2014 and is consistent with literature from other parts of the world (Stromberg et al., 2015; Taylor and Hodge, 2014).

The prevalence of Trichostrongylid-type egg positive samples found in this study is higher than the prevalence reported by Polley and Bicks (1987) of 63% in intensively run (30 cows and their calves, which were rotated every two to three weeks among a set of four to six approximately eight hectare paddocks in Saskatchewan. The prevalence of 79% in calves reported in this study is also higher than that reported by Colwell et al. (2014), who sampled weaned beef calves in 2008, 2009 and 2010 in Alberta and found a highest prevalence of 48.3%. However, fecal samples from that study were collected in one of the hottest and driest regions of Alberta and the samples were frozen prior to processing; it is possible that this may have resulted in a reduced egg recovery rate and, therefore, lower prevalence estimation. The prevalence found in calf samples was, however, similar to an extensive study of GIN prevalence in weaned beef calves from 291 herds from 24 states in the United States, which found an overall prevalence of 85.6% (Stromberg et al., 2015). The high prevalence of GIN burdens and the low FEC intensity seen in cows and replacement heifers were to be expected based on GIN epidemiology and recent studies on beef cow-calf herds in Canada (Jelinski et al., 2016; Mackie, 2016)

The prevalence and FEC intensity of GIN burdens based on the GEE were influenced by season and production type and varied similarly seasonally during the study period. The prevalence of GIN burdens in cows remained fairly constant and the FEC intensity of the burdens (EPG) remained consistently low. The prevalence and FEC intensity of GIN burdens in calves

and heifers were more variable but with the prevalence and FEC intensity of burdens increasing from spring/summer to the fall. The increase in FEC intensity from spring/summer to fall is expected as these younger and more naïve animals have prolonged exposure to infective L3 larvae on pasture during the spring/summer grazing period. In contrast, such a trend was not seen in the mature cows which was also expected as they have had repeated exposure to GIN and have developed an acquired immunity (Gasbarre, 1997).

The results of this study also correspond to the known epidemiology of common cattle GIN in temperate cattle producing regions (Gibbs, 1988; Stromberg and Averbeck, 1999; Yazwinski and Tucker, 2006). Typically, animals start the grazing season with low egg counts and lower prevalence because of the reduction in transmission over the winter. Characteristically, GIN prevalence and burden rise during the grazing season because of contamination of pasture and environmental conditions more suitable to L3 survival on pasture. Calves in this study consistently had a higher GIN prevalence in the fall compared to spring/summer. Cows acted as a source of GIN for calves through pasture contamination with a higher GIN prevalence in cows in spring/summer compared to fall. An egg rise around calving, emergence of hypobiotic stages and ingestion of overwintered larvae during the early grazing period likely contributed to pasture contamination in the spring. At the end of the grazing season, GIN prevalence and burden begin to decrease because of reduced larval development on pasture and the start of GIN hypobiosis, both which will reduce the transmission (Gibbs, 1988; Stromberg and Averbeck, 1999; Yazwinski and Tucker, 2006).

It is interesting to note that the prevalence of *Nematodirus* spp. was relatively high, particularly in calves. A similar trend has been seen in the US. Stromberg et al. (2015) found a prevalence of 18% in samples from 1,772 weaned calves six to eight months in age. *Nematodirus* spp. is a parasite of low pathogenicity unless found in high numbers in young cattle that have not developed immunity (Herlich and Porter, 1953). Several reasons for this increase might include the development of anthelmintic resistance in the parasite or the timing of the application of anthelmintic drugs in current management protocols which may favor transmission of *Nematodirus* spp. *Nematodirus* spp. eggs last well unhatched on pasture in the cooler months and only hatch in the warmer weather of the following summer period; therefore, the time of peak transmission may be missed by treatment with anthelmintic drugs applied routinely in the spring or fall (Gasbarre, 2014). Monitoring of this parasite may become important to prevent the

occurrence of clinical disease in naïve young stock because of an increased prevalence and/or ineffective treatment protocols.

There are potentially serious implications for Canadian beef production with changes in GIN prevalence, burden and the development of anthelmintic resistance. This study provides a baseline for the current prevalence of GIN burdens in western Canadian beef cow-cow herds. Unfortunately, specific epidemiological information known to affect GIN burdens in grazing cattle was not collected in this study. Useful information would have included: exact geographical location of samples to account for environmental conditions (humidity, temperature and precipitation), access to pasture/pasture types, and stocking density/pasture management. The management of beef cow-calf herds in western Canada has changed considerably since Polley and Bickis conducted their study in 1986. Changes in the western Canadian beef cow-calf industry include increasing herd sizes, increasing intensiveness of production systems, later spring calving and the implementation of low-cost overwintering feeding systems (i.e. swath and bale grazing) (Jelinski et al., 2016; Statistics Canada, 2017, 2016).

Along with the changes in beef cow-cattle management in western Canada, suspected development of anthelmintic resistance and changes in climate also need to be considered for their impact on GIN burdens in beef cattle (Fox et al., 2015; Gasbarre et al., 2009b; Morgan et al., 2013; United States Department of Agriculture, 2008). The generally high prevalence of GIN burdens seen in this study highlights the need for more detailed examination of the epidemiology of GIN on western Canadian beef cow-calf herds, taking into account the factors mentioned above. In addition, evaluation of anthelmintic efficacy and a more in-depth understanding of producers' attitudes and management approaches to GIN is needed to better understand how GIN burdens in beef cattle are best managed sustainably in the future.

Samples collected in this study represent convenience samples from beef producers who were motivated to sample these particular herds/animals and who had contact/input from MERCK Canada sales representatives; therefore, care should be taken when extrapolating the results for a wider population (Thrusfield, 2013). Furthermore, because of low sampling numbers in some seasons and production type (e.g. heifer samples in the fall of 2014), it would be of limited value to interpret results down to that level. However, the aim of the study was to describe trends in GIN prevalence and FEC intensity in cow-calf herds in western Canada more broadly, which was achieved with this study.

Limitations in this study and in most studies of GIN in cattle that must be considered include the difficulties in accurately diagnosing burden, particularly quantifying the FEC intensity of the burden. Fecal egg counts are routinely used for diagnosis; however, they have been shown to be poorly correlated with actual burden in cattle, especially adult cattle with acquired immunity (Eysker and Ploeger, 2000; Roeber and Kahn, 2014). Despite this, FEC are widely accepted as an appropriate way of monitoring GIN, particularly until a more effective alternative can be validated (Coles, 2002; Coles et al., 1992). The lack of determination of larval species identity of GIN present in the samples from this study is also important as the fecundity of adults and in fact the pathogenicity of species varies considerably (Scott and Sutherland, 2009). Future research into the epidemiology of GIN in western Canadian beef cow-calf herds should where possible address these issues by utilizing more recent technologies such as a deep-sequencing nemabiome assay and/or anti-body ELISA's in conjunction with more conventional methods such as FEC to quantify GIN infection intensity in cattle (Avramenko et al., 2015; Colwell et al., 2014).

2.6 Conclusion

This study provides a much-needed summary of gastrointestinal nematode burdens in beef cattle from cow-calf herds of the western Canadian prairie provinces. The findings support known epidemiological patterns for GIN transmission during the grazing season and the increased susceptibility of calves compared to cows. The high prevalence of positive fecal egg counts, when compared to historical data and when considering recent changes in cattle management, climate and emerging anthelmintic resistance, highlights the need for further investigations. These should include obtaining a better understanding of producers' knowledge and current management practices for GIN in their cattle, and further closing the knowledge gap on GIN prevalence, fecal intensity and species of GIN in western Canadian beef cattle. This information is necessary in order for more strategic control methods to be developed that maintain efficient production, while limiting the development of anthelmintic resistance.

2.7 Acknowledgments

We thank MERCK Animal Health Canada for the database of fecal egg counts utilized for this analysis.

2.8 TABLES

TABLE 2.1 Number of fresh environmental fecal samples collected for determination of the gastrointestinal nematode prevalence and fecal egg count intensity from beef cows, calves and heifers from western Canada between 2012 and 2014, by year and season of collection.

		Cows		Calves		Heifers		Overall total	
		#Samples	#Herds	#Samples	#Herds	#Samples	#Herds	#Samples	#Herds
2012								**1,880**	**92**
	Spring/ Summer	744	43	515	32	400	22	1,659	85
	Fall	117	3	76	3	28	2	221	7
2013								**1,361**	**70**
	Spring/ Summer	478	25	171	10	423	22	1,072	54
	Fall	83	5	161	9	45	2	289	16
2014								**651**	**39**
	Spring/ Summer	237	13	91	6	54	3	382	21
	Fall	121	10	137	10	10	1	268	18
Overall total		1780	99	980	70	960	52	**3,891**	**201**

TABLE 2.2 Final GEE model for predicted prevalence (95% confidence interval) Trichostrongylid-type egg positive samples, accounting for clustering by herd, in 3,891 beef cows, calves and replacement heifers from 201 herds from western Canada sampled between 2012 and 2014, by year and season of collection.

		Prevalence (95% CI*)			
		Cows	Calves	Replacement heifers	All
2012					**84 (79-88)** [a]
	Spring/Summer	86 (81-90)	83 (77-89)	85 (80-91)	85 (81-89)
	Fall	63 (49-76)	88 (81-96)	88 (73-100)	77 (68-85)
2013					**71 (65-77)** [b]
	Spring/Summer	74 (67-81)	70 (60-79)	73 (65-81)	73 (66-79)
	Fall	44 (31-57)	78 (67-89)	77 (74-89)	62 (52-73)
2014					**79 (72-86)**
	Spring/Summer	82 (74-89)	78 (69-88)	81 (72-90)	81 (73-88)
	Fall	55 (42-68)	85 (76-93)	84 (65-100)	71 (62-81)
All		75 (72-80) [c]	79 (74-84) [d]	81 (75-87) [d]	**78 (75-82)**
	Spring/Summer	81 (76-87) [e]	78 (71-85) [f]	80 (75-93) [f]	80 (76-84)
	Fall	55 (39-71) [f]	84 (75-93) [f]	83 (73-94) [f]	71 (63-78)

*Confidence interval

[a, b] Statistically significantly different; p=0.001

[c, d] Statistically significantly different; highest p=0.03

[e, f] Statistically significantly different; highest p=0.008

TABLE 2.3 Final GEE model for predicted mean EPG (95% confidence interval), accounting for clustering by herd, for Trichostrongylid-type eggs in 3,891 beef cows, calves and replacement heifers from 201 herds from western Canada sampled between 2012 and 2014, by year and season of collection.

		Mean EPG (95% CI*)			
		Cows	Calves	Replacement heifers	All
2012					**5.8 (4.2-7.3)** [a]
	Spring/Summer	6.2 (3.5-8.9)	7.4 (3.1-11.6)	4.3 (2.8-5.9)	6.1 (4.4-7.7)
	Fall	2.0 (1.1-2.9)	7.6 (3.6-11.6)	5.4 (1.2-9.7)	4.5 (2.7-6.4)
2013					**3.0 (2.5-3.7)** [b]
	Spring/Summer	3.8 (2.9-4.7)	2.0 (1.1-3.0)	3.7 (2.6-4.8)	3.2 (2.6-3.9)
	Fall	1.2 (0.6-1.8)	2.1 (1.1-3.1)	4.6 (1.0-8.1)	2.3 (1.3-3.3)
2014					**5.0 (2.7-7.3)**
	Spring/Summer	4.6 (2.7-6.5)	8.9 (1.8-16.0)	2.1 (0.3-3.8)	5.2 (2.6-7.8)
	Fall	1.5 (0.9-2.0)	9.2 (3.5-14.9)	2.6 (0.0-5.2)	4.0 (2.1-6.0)
All		4.4 (3.2-5.7) [c]	5.8 (3.5-8.3) [d]	3.9 (2.9-4.9)	**4.9 (3.9-5.9)**
	Spring/Summer	5.6 (3.6-6.6) [f, g]	5.8 (2.9-8.6) [f]	3.7 (2.8-4.7) [f, h]	5.1 (4.0-6.2)
	Fall	1.6 (1.0-2.2) [e]	6.0 (3.7-8.3) [f, g]	4.6 (1.2-8.1) [f]	3.8 (2.5-5.1)

*Confidence interval

[a, b] Statistically significantly different; p<0.001

[c, d] Statistically significantly different; p<0.001

[e, f] Statistically significantly different; highest p=0.02

[g, h] Statistically significantly different; highest p=0.043

TABLE 2.4 Final GEE model for predicted prevalence (95% CI) of *Nematodirus* spp. egg positive samples, accounting for clustering by herd, in 3,891 beef cows, calves and replacement heifers from 201 herds from western Canada sampled between 2012 and 2014, by year and season of collection.

		Prevalence (95% CI)			
		Cows	Calves	Replacement heifers	All
2012					**20 (16-25)** [a]
	Spring/Summer	5 (1-8)	46 (36-55)	8 (3-12)	18 (14-21)
	Fall	10 (3-12)	67 (51-83)	17 (3-30)	29 (16-41)
2013					**8 (5-10)** [b]
	Spring/Summer	1 (0.3-2)	17 (8-26)	2 (0.3-4)	6 (3-9)
	Fall	3 (0-5)	32 (20-45)	5 (1-9)	12 (7-17)
2014					**11 (5-17)** [b]
	Spring/Summer	2 (0-4)	25 (10-41)	3 (0-7)	9 (3-15)
	Fall	4 (0-9)	45 (26-64)	7 (0-15)	17 (8-26)
All		3 (1-6) [e]	36 (30-42) [f]	6 (2-10) [e]	**14 (11-17)**
	Spring/Summer	3 (1-5)	32 (25-40)	5 (2-8)	12 (10-15) [c]
	Fall	7 (0-14)	51 (39-63)	11 (2-20)	21 (13-29) [d]

[a, b] Statistically significantly different; highest p=0.04

[c, d] Statistically significantly different; p=0.02

[e, f] Statistically significantly different; p<0.01

TABLE 2.5 Final GEE model for predicted mean EPG (95% CI), accounting for clustering by herd, for *Nematodirus* spp. eggs in 3,891 beef cows, calves and replacement heifers from 201 herds from western Canada sampled between 2012 and 2014, by season and year of collection.

		Mean EPG (95% CI)			
		Cows	Calves	Replacement heifers	All
2012					**1.1 (0.6-1.7)** [a]
	Spring/Summer	0.1 (0-0.23)	1.1 (0.7-1.5)	0.1 (0-0.23)	0.4 (0.3-0.6)
	Fall	0.9 (0.1-1.7)	7.6 (2.9-12.3)	0.87 (0.1-1.6)	2.9 (1.1-4.6)
2013					**0.1 (0-0.2)** [b]
	Spring/Summer	0.0 (0-0.0)	0.1 (0-0.2)	0.0 (0-0.0)	0.0 (0-0.1)
	Fall	0.1 (0-0.2)	0.9 (0.1-1.7)	0.1 (0-0.2)	0.3 (0-0.6)
2014					**0.3 (0.1-0.5)** [b]
	Spring/Summer	0.0 (0-0.1)	0.3 (0.1-0.5)	0.0 (0-0.1)	0.1 (0.0-0.2)
	Fall	0.2 (0-0.4)	2.0 (0.5-3.5)	0.2 (0-0.5)	0.8 (0.2-1.3)
All		0.1 (0-0.2) [c]	1.2 (0.7-1.6) [d]	0.1 (0-0.2) [c]	**0.5 (0.3-0.7)**
	Spring/Summer	0.1 (0-0.1)	0.6 (0.4-0.9)	0.1 (0-0.1)	0.2 (0.2-0.2) [e]
	Fall	0.5 (0.1-0.9)	4.3 (1.8-6.8)	0.5 (0.1-0.9)	1.7 (0.7-2.7) [f]

[a, b] Statistically significantly different; $p \leq 0.001$

[c, d] Statistically significantly different; $p < 0.001$

[e, f] Statistically significantly different; $p < 0.001$

CHAPTER THREE

GASTROINTESTINAL NEMATODE PREVALENCE, FECAL EGG COUNT INTENSITY AND LARVAL SPECIES IDENTITY FROM COW-CALF HERDS IN THE WESTERN CANADIAN PRAIRIE PROVINCES

Felicity K. Wills[1]; John R. Campbell[1]; Cheryl L. Waldner[1]; Sarah E. Parker[1]; Fabienne D. Uehlinger[1].

[1]From the Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK, S7N 5B4, Canada.

This chapter represents the analysis of data collected from a pilot disease surveillance network (Western Canadian Cow-Calf Surveillance Network), established as a five-year pilot study to provide for the systematic collection of data from the western Canadian beef cow-calf industry. Fecal samples were collected from twenty randomly selected heifers (or where 20 heifers were not available, the youngest bred cows) between September 2016 and February 2017 from 85 beef cow-calf herds from the western Canadian prairie provinces. Fecal egg counts, coproculture and determination of larval species identity was performed on these samples. This chapter represents a continuation of the information analysed in chapter 2. The aim was to provide description of gastrointestinal nematode prevalence, FEC intensity and species composition from more systematically sampled herds and to include determination of larval species identity which was not conducted previously and which is important for the interpretation of fecal egg count results, management and treatment choices. The results of this chapter revealed consistently high prevalence of gastrointestinal nematodes in these herds, indicating that further research into the current epidemiology, production effects and management of gastrointestinal nematode burdens on beef cow-calf herds of the western

Canadian prairie provinces are warranted. The predominant species of gastrointestinal nematodes identified were Ostertagia ostertagi and Cooperia punctata. This determination of larval species identity information is the first reported proportional determination of larval species identity of gastrointestinal nematodes reported for western Canadian beef cow-calf herds, utilizing a new molecular technology that involves deep amplicon sequencing of the IST-2 locus of the nematodes ribosomal deoxyribonucleic acid. Results also showed some differences in gastrointestinal nematode burden based on herd size. The next chapter of this thesis involved the development of a questionnaire to gather information about current management practices and producer opinions of gastrointestinal nematodes. This information may allow the identification of risk factors for gastrointestinal nematode burdens in western Canadian beef cow-calf herds.

AUTHOR CONTRIBUTIONS: Uehlinger, Campbell and Waldner were responsible for design of the data analysis and manuscript review. Wills was responsible for design and analysis of data collection and analysis of samples and manuscript preparation.

CONTRIBUTIONS: Molecular diagnostics were performed by Drs. John Gilleard, Elizabeth Redman and Russell Avramenko, and bioinformatic analysis of raw sequence data was performed by Dr. Elizabeth Redman at the University of Calgary, Alberta. Fecal sample laboratory processing was performed by Felicity Wills, Sam Ekanayake, Taylor Gibson and Kathleen Holweger.

3.1 Abstract

Subclinical gastrointestinal nematode burdens in grazed beef cattle can cause reduced production efficiency. Currently there is a lack of information about gastrointestinal nematode burdens in beef cow-calf herds in western Canada. Samples (n=1,655) were collected form 20 randomly selected heifers (n=85 herds), from late September 2016 to February 2017. A saturated sugar flotation and double centrifugation technique, along with larval coproculture and deep amplicon nemabiome sequencing was used to determine gastrointestinal nematode prevalence, FEC intensity, species identity and burden composition. The unadjusted predicted prevalence of Trichostrongylid-type egg positive samples was 92% (95% CI, 89-95). The prevalence of *Nematodirus* spp. (1.8%; 95% CI 1.3-2.6) and *Trichuris* spp. (1.2%; 95% CI 0.8-1.9) was very low. The unadjusted predicted mean egg per gram of feces (EPG) of Trichostrongylid-type eggs was 5.0 (95% CI 4.4-5.9). Herds with >300 cow-calf pairs had lower ($p<0.01$) predicted mean Trichostrongylid-*type* EPG at 5.0 EPG (95% CI 3.6-6.5) compared to herds with ≤300 cow-calf pairs (5.3 EPG; 95% CI 4.4-6.2). The predominant species present were *Ostertagia ostertagi* and *Cooperia punctata*. The prevalence of gastrointestinal nematode burdens in beef cow-calf herds from western Canada was high (92%) and similar to those reported recently in the northern United States (85.6%). The significant difference in FEC intensity of Trichostrongylid-type eggs between herds sizes highlights that management practices may influence the level of FEC intensity. Emerging anthelmintic resistance and the need for evidence-based sustainable internal parasite control practices, further investigations into the epidemiology and parasite management practices are warranted.

3.2 Introduction

Information is currently lacking about the epidemiology of gastrointestinal nematode (GIN) burdens in beef cow-calf herds from western Canada. With an increasing world population, there is growing demand for beef. The sustainable growth of beef production in Canada is reliant on the development and adoption of more efficient management practices and technologies (Legesse et al., 2016). For beef cow-calf producers this means continued optimization of feed conversion efficiency which results in improved growth and reproductive performance (Hawkins, 1993).

Gastrointestinal nematodes in cattle can have a substantial detrimental effect on production (Kunkle et al., 2013; Reinhardt et al., 2006). Stromberg & Gasbarre (2006) and Grisi et al. (2014) examined the economic losses caused by GIN in cattle in the US and Brazil, respectively, and found them to be between two and seven billion dollars annually. There is no current information on the cost of production losses because of GIN in western Canada (Gilleard, 2016).

Important factors for control and diagnosis, such as fecundity and pathogenicity, vary with species and GIN prevalence, burden and species composition vary with geographical location and from herd-to-herd. Historically, the most prevalent GIN species found in south eastern Canada were *Ostertagia ostertagi* and *Cooperia oncophora* (Ranjan et al., 1992; Slocombe and Curtis, 1989). However, there is a paucity of literature that looks at the current prevalence and FEC intensity or predominant GIN species in beef cow-calf herds in western Canada. Although it is conceivable that predominant species are similar to other parts of Canada and similar temperate climates, changes in beef management in recent years, including increased herd sizes, later spring calving, greater use of winter swath or bale grazing and changing climates may have resulted in altered species compositions in infected cattle (Beaulieu, 2015; Fox et al., 2015; Gethings et al., 2015; Jelinski et al., 2016; Statistics Canada, 2014; Stromberg and Averbeck, 1999; Yazwinski and Tucker, 2006). Knowing the predominant GIN species present is important in interpreting diagnostics results, in the selection of anthelmintic drugs, for determining timing of application, and when making decisions that may help to limit the development of anthelmintic resistance (Sutherland and Leathwick, 2011).

To employ economical and practical control strategies it is important to understand the epidemiology of GIN in western Canadian beef cow-calf herds. Other countries including the

United States have developed integrated information gathering systems, such as the National Animal Health Monitoring System (NAHMS), which allows for the uniform and systematic gathering of data for timely comparisons in animal health and management factors between years and geographical regions (USDA, 2010). A similar monitoring network was recently established in western Canada with beef cow-calf producers. The Western Canadian Cow-Calf Surveillance Network (WCCCSN) is comprised of approximately 120 beef cow-calf herds. Enrolled producers committed to participate in a five-year program that was set up to collect information on herd demographics, management systems and disease surveillance, including determination of GIN prevalence, FEC intensity and determination of species identity and composition.

Current gaps in information regarding the epidemiology of GIN burdens in beef cow-calf herds in western Canada, meant the objectives of this study within the WCCCSN were: 1) to describe the current prevalence of GIN burdens in heifers from beef cow-calf operation from the western Canadian prairie provinces; 2) to define the current FEC intensity of GIN burdens in heifers from beef cow-calf operation from the western Canadian prairie provinces; 3); and to determine whether GIN prevalence and FEC intensity differed based on herd size, age and body condition of sampled cattle, 4) to describe the current species compositions of GIN burdens in heifers from beef cow-calf operation from the western Canadian prairie provinces.

3.3 Materials and methods

3.3.1 Sample population

The recruitment process of producers into the WCCCSN has been previously described (Moggy et al., 2017; Waldner et al., 2017). Briefly, recruitment of producers into the WCCCSN was based on the 2011 Canadian Agricultural census and was aimed at creating a geographically representative sample of cow-calf enterprises in the three prairie provinces (Alberta, Saskatchewan and Manitoba) (Statistics Canada, 2011). Producers were recruited through their regular herd veterinarians. Recruitment was contingent on a willingness to participate in questionnaires, biological sample collection and a minimum herd size of 70 cows. At the time of data collection for this study, there were 111 herds enrolled in the WCCCSN (55 in Alberta, 35 in Saskatchewan and 21 in Manitoba).

Information about herd size was derived from a questionnaire distributed to these same producers in the summer of 2016 and which asked specifically about the 2015 grazing period and herd size in that year. At the time of fecal sample collection, the month of collection, the age and body condition score (BCS) of each sampled animal was recorded and submitted together with the fecal sample.

3.3.2 Fecal sample collection

Fecal sampling began in late September 2016 and continued until February 2017. At pregnancy checking by the routine herd veterinarian, fresh fecal samples were taken from the rectum of 20 randomly selected heifers, where available; if 20 heifers were not available, the youngest bred cows were to be sampled for a total of 20 samples per herd. The number of heifers sampled per herd was made based on current recommendations in the literature (Gasbarre et al., 1996; Wood et al., 1995). Heifers were chosen to be sampled as they are more susceptible than cows to production and reproduction losses as a result of appetite suppression, digestive disturbance and hormonal imbalance caused by GIN burdens (Fox, 1997). Their increased susceptibility is because of their incomplete development of acquired immunity to GIN and also stress that gestation places on them (Loyacano et al., 2002). A fresh rectal glove was used for each animal. Feces were placed into individually labeled whirl packs and all air was expelled to create an anaerobic environment. Samples were stored at room temperature prior to shipping and were sent by courier to the laboratory (University of Saskatchewan) within 24 hours of collection.

3.3.3 Fecal egg counts

Individual fecal samples were processed within 72 hours of collection using a saturated sugar flotation and double centrifugation technique. In brief, five grams of feces were mixed with 12ml of water to create a fecal slurry. This slurry was strained through cheesecloth (grade 60) into a test tube, which was centrifuged (relative centrifugal force (RCF) 180 x g) at 1500 rotations per minute (rpm) for 10 minutes. The supernatant was then decanted and the sediment reconstituted with a saturated sugar solution (specific gravity 1.27). The samples were again centrifuged at 1500 rpm for 10 minutes. Using the saturated sugar solution, a slight convex meniscus was created on the test tube and a cover slip placed on top. Samples were then left to

stand for at least 30 minutes. The cover slip was removed and placed on a microscope slide for examination at 40x magnification. These flotation and centrifugation methods have been shown by Egwang and Slocombe (1981) to detect samples with egg fewer than seven eggs per gram. Gastrointestinal nematode eggs were identified based on morphology as Trichostrongylid-type, *Nematodirus* spp. or *Trichuris* spp. and reported as eggs per five grams of feces (EP5G). Eggs per gram of feces (EPG) was calculated by dividing the egg counts by the original weight of the sample.

3.3.4 LARVAL COPROCULTURE

For subsequent GIN species identification, a coproculture protocol for isolation of third stage nematode larvae (L3) was employed. Briefly, for each herd, 12g of feces from each individual animal was pooled and mixed well to create a composite sample. Eighty grams of the pooled fecal sample was mixed with an equal volume of vermiculite and wetted with water in a clean culture glass. Cultures were moistened with water every 48 hours or as needed for 21 days at room temperature (approx. 20^{o}C), after which the culture glass was filled with warm water, covered with a petri dish and inverted. The petri dish was then filled with warm water and left to stand for 24 hours to allow hatched larvae to migrate out of the culture into the petri dish. The contents of the petri dish were then transferred to a 15mL falcon tube and centrifuged at 4000rpm for three minutes. The remaining supernatant was then decanted and the pellet re-suspended in a small volume of water and centrifuged at 4000 rpm for three minutes, for two cycles. The resulting pellet was re-suspended in 1mL of 70% ethanol. Samples were stored at 4°C until processing for determination of larval species identity. This process was repeated in triplicate for each herd.

3.3.5 DETERMINATION OF LARVAL SPECIES IDENTITY

A deep amplicon sequencing assay of the ITS-2 region of the nematodes' rDNA was used to identify the nematode species present and their relative proportions. Third-stage larvae were pooled by province and herd size into three composite samples each of 200 L3s and 1000 L3s, resulting in six larval pools for each province and herd size for a total of 36 samples. This assay has been previously described and validated by Avramenko *et al*., (2015). Briefly, larvae

were placed in a Proteinase K (120 μg/mL) lysis buffer (50 mM KCl, 10 mM Tris (pH8.3), 2.5 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20, 0.01% (w/v) gelatin) to create pooled crude lysates. Molecular grade water (1:10) was used to dilute the pooled crude lysates. The diluted pooled crude lysates were used as template for the amplification of the ITS-2 region. The manufacturers protocol was followed to purify the PCR products with AMPure XP Magnetic Beads (1X) (Beckman Coulter, Inc.). The resulting rDNA ITS-2 amplicons had Illumina indices and P5/P7 sequencing tags added using limited cycle PCR amplification. Products then underwent the same purification process described above. Approximately ~50ng of the resulting products were pooled to make up the master sequencing library. The KAPA qPCR Library Quantification Kit (KAPA Biosytems, USA) was used to get the final concentration of the pooled library. At a concentration of 12.5 nM with the addition 25% PhiX Control v3 (Illumina, FC-110-3001) and using a 500-cycle pair-end reagent kit (MiSeq Reagent Kits v2, MS-103-2003) the pooled library was run on an Illumina MiSeq Desktop Sequencer. The bioinformatic pipeline utilized was described in depth by Avramenko *et al*., (2015). Firstly consensus sequences were generated from raw overlapping pair-end FASTQ sequences. Samples with <2000 reads, were discarded, as this is indicative of a failed sample preparation. Consensus sequences were searched against a database of reference sequences using BLASTIN. BLASTIN was generated from the sequencing of the rDNA ITS-2 sequence from single larvae derived from monoculture experimental burdens. An identity threshold of >97% was required to allocate each sequence to a species reference. This allowed for the accommodation of sequencing errors and intra-species variation in the ITS-2 region. The GenBank database was used to assign species that did not meet the >97% threshold. Sequences that did not hit any rDNA ITS-2 sequence in GenBank were discarded as artifacts or contaminating sequences. The number of raw reads per species identified was divided by the number of raw reads to determine the percentage species composition of each sample.

3.3.6 DATA ANALYSES

The information collected at the time of sampling and the fecal egg counts were entered into a commercial spreadsheet program (Excel 2013; Microsoft Corp., Redmond, Washington,

USA) for data checking and imported into a statistical software package (StataSE version 14, Stata, College Station, Texas, USA).

Herd size was based on the maximum number of cow-calf pairs run by the herds in the 2015 grazing period. Herds were classified as ≤300 cow-calf pairs or >300 cow-calf pairs. Based on age of cattle, samples were categorized: either 12-23 months old or 24-36 months old. Samples from cows >36 months and <12 months old or where age was not reported were not included in the analyses. The BCS was graded on a scale of 1 to 5 and categorized as BCS ≤2.5 and BCS >2.5 (Hess et al., 2005; Stromberg et al., 1997). Samples were further classified by month of sample collection (September/October, November/December and January/February). The number of fecal samples submitted was summarized by herd size, age, BCS and month of sample submission (Table 3.1).

Raw descriptive summary statistics including prevalence (and exact binomial 95% confidence interval (CI)) of positive samples were performed for *Trichostongylid*-type egg, *Nematodirus* spp. and *Trichuris* spp. Fecal egg counts per gram of feces (EPG) for *Trichostongylid*-type egg, *Nematodirus* spp. and *Trichuris* spp. were depicted as geometric mean (±SD) and range. Geometric means were used because of the over-dispersed nature of the FEC data. The very low prevalence of *Nematodirus* spp. and *Trichuris* spp. meant further analysis was restricted to Trichostrongylid-type eggs only.

The overall prevalence (95% CI) of Trichostrongylid-type eggs was estimated using generalized estimating equations (GEE) to account for clustering within herds. The initial null or intercept only GEE model used a binomial distribution and logit link function with an exchangeable within-group correlation structure and robust standard error (to deal with overdispersion within the data). The overall mean Trichostrongylid-type EPG (95%CI) was also determined using a null GEE model with a negative binomial family and log link with an exchangeable within-group correlation structure and robust standard error.

The effects of herd size, age, BCS and month of sample submission on the Trichostrongylid-type prevalence and EPG burdens were further assessed with fixed effects introduced in the above GEE models. Each independent variable (herd size, age, BCS and month of sample submission) was forced into the final model and plausible interaction terms (herd size by age, herd size by BCS, herd size by month of submission, age by BCS and herd size by age by BSC) were evaluated. The final GEE model for each outcome was produced by manual stepwise

backwards elimination. Interaction terms were retained in the GEE if found to be a statistically significant based on a Wald's test at a p-value of ≤0.05. The effect of retained predictor variables on the predicted prevalence of Trichostrongylid-type positive samples and predicted mean EPG were assessed using post-hoc pairwise comparison with a level of significance set at p-value ≤0.05.

The results of the determination of nematode species identity were calculated by dividing the number of species specific reads obtained from each sample and dividing those by the total number of reads obtained from that samples. The relative proportions of each nematode species were reported with the corresponding geometric strongylid FEC and estimated starting number of L3.

3.4 RESULTS

3.4.1 SAMPLE POPULATION AND NUMBER OF SAMPLES COLLECTED

During fall pregnancy diagnosis in 2016, 1,849 fecal samples were collected from 93 herds. After omission of samples from heifers <12 months and cows >36 months or with no age recorded (n=195), 1,655 samples from 85 herds were included in the analysis. Table 3.1 shows the number of samples by herd size, age, BCS and month of sample collection. The number of samples collected from each operation ranged from 6 to 20 samples (mean 19.5, standard deviation (SD) 2.0).

Most (65%) samples came from herds with less than 300 cows, from cattle <24 months old (60%), and from animals with a BCS >2.5 (91%). Month of submission ranged from September 2016 until February 2017 with the largest proportion (65%; 1,069/1,655) of samples collected in November and December 2016.

3.4.2 RAW AND UNADJUSTED PREVALENCE AND FECAL EGG COUNT OF TRICHOSTRONGYLID-TYPE EGGS

Most of the GIN eggs detected in submitted samples were Trichostrongylid-type eggs with 1,522/1,655 (92%; 95% CI 91-93) samples positive (Table 3.1). The raw geometric mean Trichostrongylid-type EPG was 3.0 (SD 3.7) (Table 3.1). There was very little variation in the raw prevalence of Trichostrongylid-type egg positive samples between different herd size, ages,

BCS or months of sample submission (range of 90% in samples submitted in November/December to 96% in samples submitted in September/October). Similarly, there was also very little variation in the mean EPG between the different predictor categories.

The unadjusted predicted prevalence (95% CI) from the null GEE model of Trichostrongylid-type egg positive samples was 92% (95% CI 89-95). The unadjusted predicted mean EPG of Trichostrongylid-type eggs was 5.0 (95% CI 4.4– 5.9).

3.4.3 Raw prevalence and fecal egg count of Nematodirus spp. and Trichuris spp.

Nematodirus spp. was an infrequent finding with 30/1,655 (1.8%; 95% CI 1-3) of samples positive. The *Nematodirus* spp. positive samples came from 21 herds with a maximum of 3/20 animals per herd positive. The raw geometric mean EPG was 0.3 (SD 2.0) with a range of 0-2.4 EPG.

Trichuris spp. was found even less frequently with only 1% (95% CI 1-2; 20/1,655) of all samples positive. The raw geometric mean EPG was 0.2 (SD 1.5) with a range of 0-0.6 EPG. The *Trichuris* spp. positive samples came from 18 herds, with a maximum of 2/20 animals per herd positive.

3.4.4 Final GEE for the predicted prevalence of Trichostrongylid-type eggs

After the stepwise backward elimination of non-significant interactions, the final GEE model included herd size (p=0.98), age (p=0.47), BCS (p=0.90) and month of submission (p=0.12) (Appendix Table A3.1). None of the fixed effects or tested interaction terms were statistically significant. In the pairwise comparisons, samples submitted in September/October had a significantly (p=0.045) higher Trichostrongylid-type egg prevalence (95%; 95% CI 93-98) than those submitted in November/December (90%; 95% CI 87-94). These samples also accounted for the highest and lowest prevalence identified in this model with all other prevalence estimates falling within the range of 90 to 95%.

3.4.5 Final GEE for the predicted mean Trichostrongylid-type egg per gram of feces

After the stepwise backward elimination of non-significant interaction terms, the final GEE for the predicted mean EPG of Trichostrongylid-type eggs included herd size (p<0.01), age

(p=0.48), BCS (p=0.43) and a significant interaction between herd size, age and BCS (p<0.01) (Table A3.2). The non-significant fixed effect was month of sample submission (p=0.31).

Herds with >300 cow-calf pairs had a significantly (p<0.01) lower mean predicted Trichostrongylid-type EPG at 5.0 EPG (95% CI 3.6-6.5) compared to herds with ≤300 cow-calf pairs (5.3 EPG; 95% CI 4.4-6.2). When the significant three-way interaction between herd size, age and BCS was examined heifers aged 12 to 23 months from herds with >300 cow-calf pairs with a BCS ≤2.5 had the lowest predicted mean Trichostrongylid-type EPG at 1.8 (95% CI 1.3-2.3). The predicted mean EPG of Trichostrongylid-type eggs in these heifers was statistically significantly lower than that of heifers from all other age, BCS and herds size combinations (p<0.01) (Figure 3.1). In contrast, heifers aged 12 and 23 months with a BCS ≤2.5 from herds with ≤300 cow-calf pairs had the highest predicted mean Trichostrongylid-type EPG at 6.6 (95% CI 5.3-7.8) (Table 3.2).

3.4.6 Determination of larval species identity (ITS-2 deep sequencing assay)

Third stage larvae were harvested from 90 herds. Table A3.3 describes the samples utilized for the deep amplicon sequencing assay for the ITS-2 rDNA locus used for determination of larval species identity and gastrointestinal nematode burden composition. In all of the 36 samples submitted there was successful amplification of the rDNA from the internal transcribed spacer-2 (ITS-2) of the larvae.

The species composition as a percentage of the total number of L3 larvae in the sample and in relation to the pooled mean fecal egg counts are depicted in Figure 3.2. The nematode species found in this study were *Ostertagia ostertagi* detected in 100% of the samples, *Cooperia oncophora* (100%), *Cooperia punctata* (100%), *Haemonchus placei* (97%), *Oesophagostomum radiatum* (94%) and *Trichostrongylus* spp. (89%). Overall, *Ostertagia ostertagi* was the predominant species accounting for >50% of the L3 in 67% (24/36) of samples; it was also the most common species in all the samples from AB and SK. The proportion of *O. ostertagi* larvae in samples ranged from 21 to 65%. *Cooperia punctata* was the next most predominant species at 28% (10/36) of all samples. The proportion of *C. punctata* in samples ranged from 1 to 64%. Although *C. punctata* was present to some extent in all samples, it was the predominant species in MB only where 10/12 samples contained >50% *C. punctata*. The proportion of *C. punctata*

found in both Alberta and Saskatchewan were appreciably lower. Although not the predominant species in any sample, *Cooperia oncophora* was seen in higher proportions (range 4-41%) than *Haemonchus placei* (range 0-6%), *Oesophagostomum radiatum* (range 0-16%) and *Trichostrongylus* spp. (0-4%). Although small numbers of *Nematodirus* spp. and *Trichuris* spp. were identified morphologically in samples from this study, neither of these species were detected using the nemabiome-sequencing assay.

3.5 Discussion

The results of this study represent the most current description of the prevalence, FEC intensity and species composition of GIN in breeding heifers from the western Canadian prairie provinces. As could be expected in pasture grazed cattle the overall prevalence of GIN was high. The prevalence of Trichostrongylid-type eggs (92%) was appreciably higher than that of *Nematodirus* spp. (2%) and *Trichuris* spp. (1%). This fits with previous literature, as historically the most common (*O. ostertagi* and *C. oncophora*) and the most pathogenic (*O. ostertagi*) species of GIN in grazed beef cattle are reported to be Trichostrongylid-types (Stromberg et al., 2015; Stromberg and Averbeck, 1999).

The prevalence of Trichostrongylid-type egg positive samples here was higher than the 63% prevalence reported by Polley and Bicks (1987) in intensively run cows in Saskatchewan. However, a FEC technique (McMasters) with a higher limit of detection was used in that study which could contribute to the difference seen in prevalence. It is also likely that differences in the sampled populations contribute to these differing results. In the study here, the focus was on sampling heifers as opposed to more mature cows which was the case in the study by Polley and Bicks. The high GIN prevalence found in cattle from this study was similar to that seen in recent studies in beef cow-calf herds in the United States which reported a prevalence of strongyle type eggs of 85.6% (Stromberg et al., 2015). Although animals sampled in this study were weaned calves.

The FEC intensity of GIN as described by the Trichostrongylid-type EPG counts in this study were consistently low across all evaluated variables. As with the Trichostrongylid-type egg prevalence, the level of FEC intensity is consistent with what has been reported for grazed beef cattle in Canada. A recent study conducted by Mackie (2016) on a beef cow-calf operation in

Ontario found consistently low FEC in pasture grazed cows, with a peak of 4 EPG. Jelinski et al. (2016) also reported a consistently low level of FEC intensity in grazed beef cows in Saskatchewan in 2014.

Examination of Trichostrongylid-type egg EPG with GEE revealed a significant difference in the predicted mean EPG based on herd size. The significant interaction in the predicted mean EPG of Trichostrongylid-type eggs identified by the GEE model was also influenced by herd size. The difference in predicted mean EPG based on herd size may indicate that differing management practices exist between larger and smaller beef cow-calf herds and that these management practices affect GIN burden. There are many management factors that can influence GIN burdens including pasture type/quality, grazing management (stocking density), management of animal production type and use of anthelmintics (Yazwinski and Tucker, 2006). While the difference in EPG counts was very small in this study and most likely not clinically significant it does highlight the need for further research to be carried out looking at the current management strategies utilized by cow-calf producers.

It must also be noted, however, that while the low level of GIN FEC intensity seen here would unlikely have been high enough to cause clinical disease, some level of subclinical disease would be expected. Subclinical GIN burdens are associated with reduced production efficiency in beef cattle, largely attributable to reduced feed intake and reduced nutrient absorption (Forbes et al., 2002; Fox, 1997). In the northern US, Kunkle et al. (2013) reported that otherwise healthy, young grazing cattle treated for GIN gained an average of 20kg more than their untreated counterparts. While the GIN FEC intensity in both treated and untreated (co-grazed) cattle decreased over the grazing season in that study, the mean geometric EPG in some of the untreated control groups at the end of the grazing season were similarly low (e.g. 2, 4 and 8 EPG) as in this study here, highlighting that even a low GIN FEC intensity can limit production. It must also be considered that low burdens in breeding stock like cows and heifers act as a source of pasture contamination that could directly impact the level of burden acquired by naïve young stock (Forbes et al., 2002).

Fecal egg counts are commonly used for the diagnosis of GIN burden in cattle; however, they have limitations in their ability to accurately quantify (particularly in adults) GIN burden in grazed cattle. Factors that affect the accuracy of FEC and our ability to interpret the results include species mix (resulting in different fecundity and pathogenicity), environmental conditions

(temperature, humidity and precipitation), immune status/health of the host and season of sampling.

Other factors that may contribute to the low FEC seen in grazing cattle include the aggregation of eggs within fecal samples from groups and the dilution of eggs within a large volume of feces (Eysker and Ploeger, 2000; Yazwinski and Tucker, 2006). The use of FEC in older cattle has been considered to be of limited value as a diagnostic tool. However, a series of repeated FEC from a number of animals may offer more useful information at the herd level (Gasbarre et al., 1996; Yazwinski and Tucker, 2006). Although FEC was considered an appropriate diagnostic tool for this study, due caution is warranted in their interpretation.

Avramenko (2015) validated a novel technique for determination of larval species identity utilizing a deep sequencing assay of the ITS-2 region of the nematodes' ribosomal deoxyribonucleic acid. This technique allows for the species specific proportional diagnosis of mixed GIN burdens. The ITS-2 rDNA deep-sequencing nemabiome assay has a high analytical specificity, and is able to detect even a single larvae in a mixed sample (Avramenko et al., 2015). This method does not provide any information, however, on the level of GIN burden; it only provides the relative proportions of different species in the sample. The advantage of using the next generation sequencing technology is the ability to determine the relative quantification of species in a sample, thus making the interpretation of FEC more useful. For example, high FEC from samples with high proportions of very fecund GIN (e.g. *Cooperia* spp.) may be less concerning than high FEC from samples with a high proportion of less fecund GIN (*Ostertagia* spp.), as it would suggest lower numbers of adult GIN. This would be the same when the differing pathogenicity of GIN is considered.

In the samples, here *O. ostertagi*, considered the most pathogenic GIN seen in beef cattle, was identified in 100% of the samples and it was found in the highest concentration in 67% of samples. Interestingly, *C. punctata* was also found in 100% of samples and was the predominant species in 28% of them. Historically *C. punctata* has not been diagnosed as frequently in mixed GIN burdens in beef cattle as the less pathogenic *C. oncophora*. *Cooperia oncophora* was also found in all samples in this study but in lower proportions, and, together with *Ostertagia* spp. is considered one of the most common GIN found in beef cattle (Ranjan et al., 1992; Slocombe and Curtis, 1989; Stromberg and Corwin, 1993).

Recent reports from the US, however, have shown a rise in *C. punctata* in beef herds, particularly in single species burdens or in burdens where *C. punctata* is the prominent burden (Stromberg et al., 2012). This is particularly concerning as some of the first documented cases of anthelmintic resistance in cattle in the US over 10 years ago were seen mainly against the macrocyclic lactones with *C. punctata* having the highest survival rate following treatment (Gasbarre et al., 2009a, 2009b). *Cooperia punctata* has also been shown to reduce the average daily gain and dry matter intake in experimentally infected calves (Stromberg et al., 2012). In combination, the frequency of *C. punctata* and its predominance in some samples here is concerning as it may indicate an increasing population of macrocyclic lactone resistant GIN that have demonstrated negative production impacts.

It is interesting to note that although *C. punctata* was identified in 100% of samples, the 10 samples in which it was the predominant species were all from Manitoba. This could indicate that certain management strategies (e.g. deworming protocols) differ in herds from Manitoba or that environmental and climate conditions in that province favor the development and survival of *C. punctata*. There has been some evidence to suggest that the development of ivermectin resistance in another *Cooperia* species, *Cooperia oncophora*, was linked to increased pathogenicity. Future research into a similar event occurring with *C. punctata* would be beneficial to gain a better understanding of the potential risks associated with this particular parasite

A limitation to this study was also that it only sampled one animal production type. Future research into the epidemiology of GIN burdens in herds should include the longitudinal sampling of multiple animal production types (such as cows, calves, stocker cattle) over a whole year and include the determination of larval species identity of these samples. The longitudinal sampling of different animal production types throughout the year will increase the opportunity to elucidate the peak transmission times for each animal production type. When a single sample is taken these peak risk, points can be missed. The longitudinal sampling also would allow FEC results to be linked more closely to meteorological and management data to further identify potential risk factors for GIN burdens.

The timing of sampling of heifers is another limitation of this study. The largest proportion of samples (64%) were collected in November and December 2016. Studies by Ranjan et al (1992) in Quebec found that FEC counts for grazing cattle peaked in May and early June

and then began to decline into fall and winter. This decline in FEC is in some part attributed to the onset of hypobiosis in some GIN, like *Ostertagia ostertagi*, during the winter months. Therefore, sampling of these heifers during the fall may not have been ideal in identifying those peak levels of GIN burden and may have resulted in an underestimation of the FEC intensity. Ideally, these heifers and other animal production types would be sampled throughout the year in order to better document the epidemiology of GIN specific to beef cow-calf herds of the western Canadian prairie provinces.

3.6 Conclusion

The results of this study provide needed epidemiological information about the prevalence, FEC intensity and species composition of GIN specific to western Canadian beef cow-calf herds, which is an important sector of the Canadian agrarian economy. It confirms that the prevalence of GIN in grazed beef cattle was high while the FEC intensity was generally low. While the predominant species in the samples were *Ostertagia* spp. and *Cooperia* spp., the frequent finding of *C. punctata* and its predominance in some of the samples from only one province was somewhat unexpected. It warrants further investigations into the potential reasons for the geographical differences. The findings may also suggest underlying macrocyclic lactone resistance in many of these cattle or at least should raise concern about potential production impacts which to date have not been well characterized. Another interesting conclusion from this study was the effect that herd size apparently played in the GIN FEC intensity. More in-depth assessment of management factors between different herds would be indicated to better evaluate the potential reasons for these differences seen.

Going forward, the high prevalence of GIN and the differences seen in FEC intensity and species between herds and provinces, respectively, emphasize the need for continuing research into this production limiting disease. Several areas of concern to be addressed would be the development of more accurate methods of diagnosing burden, particularly in adult beef cattle. Obtaining more information about the current management practices of GIN in beef cattle is crucially important to gain a better understanding of potential risk factors. In combination, continued investigations are necessary to provide western Canadian beef producers with evidence-based management strategies aimed at limiting the risk of anthelmintic resistance

development and increasing the knowledge about the different (potentially changing) parasite populations and their effects on production.

3.7 ACKNOWLEDGEMENTS

We thank Drs. John Gilleard, Elizabeth Redman and Russell Avramenko from the University of Calgary, Alberta, for the molecular diagnostics were performed and bioinformatic analysis of raw sequence data.

3.8 TABLES

TABLE 3.1 Number (%) of samples collected and raw descriptive summary of Trichostrongylid-type prevalence (95% confidence interval) and mean egg counts (EPG) in fresh fecal samples (n=1,655) collected from cows and heifers at fall pregnancy diagnosis in 2016 from beef herds (n=85) in western Canada, overall and by herd size, age, body condition score (BCS) and time of collection.

		# samples	# herds	% samples	Prevalence (95% CI*)	Geometric mean (±SD^)	Median (IQR$)	Range
Overall		1,655	85	1.00	92 (91-93)	3.0 (3.7)	2.8 (5.6)	0-92
Herd size								
	≤ 300	1,080	56	65	92 (90-94)	3.1 (5.5)	3.0 (6)	0-85
	> 300	575	29	35	92 (89-94)	2.8 (5.1)	2.4 (5.2)	0-92
Age								
	< 24 months	1,001	52	60	93 (91-94)	3.2 (5.5)	3.2 (6.2)	0-92
	24- 36 months	654	43	40	91 (88-93)	2.6 (4.3)	2.6 (5.9)	0-61.4
BCS								
	≤ 2.5	154	24	9	94 (89-97)	3.0 (4.8)	2.8(5.2)	0-55.8
	> 2.5	1,501	83	91	92 (90-93)	3.0 (4.7)	2.8 (5.6)	0-92
Month of submission								
	Sep-Oct	388	20	23	96 (93-97)	3.4 (3.4)	3.6 (5.1)	0-40.2
	Nov-Dec	1,069	55	65	90 (88-91)	2.8 (5.4)	2.6 (45.7)	0-92
	Jan-Feb	198	10	12	94(90-97)	3.2 (3.3)	3.0 (6.0)	0-28

*Confidence interval, ^Standard deviation, $Interquartile range

TABLE 3.2 The predicted mean Trichostrongylid-type EPG (95% CI) for the 3-way interaction between herd size, age and BCS from the final negative binomial GEE model with an exchangeable correlation structure, a log link function and robust standard errors, in fecal samples from 1,655 heifers from 85 herds from the western Canadian prairie provinces collected in the fall of 2016.

	Predicted mean EPG (95% CI)			
	12 to 23 months		24 to 36 months	
	≤2.5 BCS	>2.5 BCS	≤2.5 BCS	>2.5 BCS
≤300 cow-calf pairs	6.6 (5.3-7.8) [b]	6.1 (4.8-7.4) [b]	5.5 (3.0-8.0) [b]	3.9 (2.8-4.9) [b]
>300 cow-calf pairs	1.8 (1.3-2.3) [a]	6.1 (3.5-8.8) [b]	4.2 (3.3-5.0) [b]	4.1 (2.8-5.4) [b]

[a, b] Statistically significantly different $p<0.001$

3.9 FIGURES

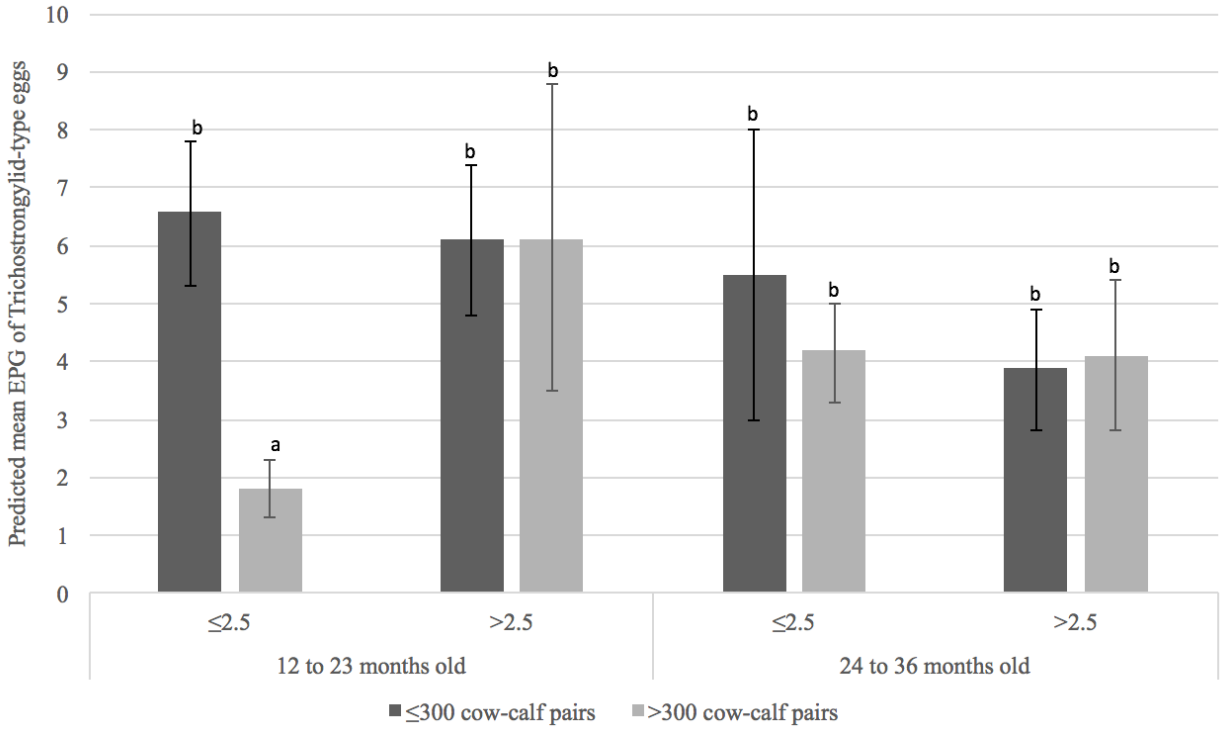

FIGURE 3.1 The predicted mean Trichostrongylid-type EPG (95% CI) for the 3-way interaction between herd size, age and BCS from the final negative binomial GEE model with an exchangeable correlation structure, a log link function and robust standard errors, in fecal samples from 1,655 heifers from 85 herds from the western Canadian prairie provinces collected in the fall of 2016.

[a, b] Statistically significantly different; $p<0.01$.

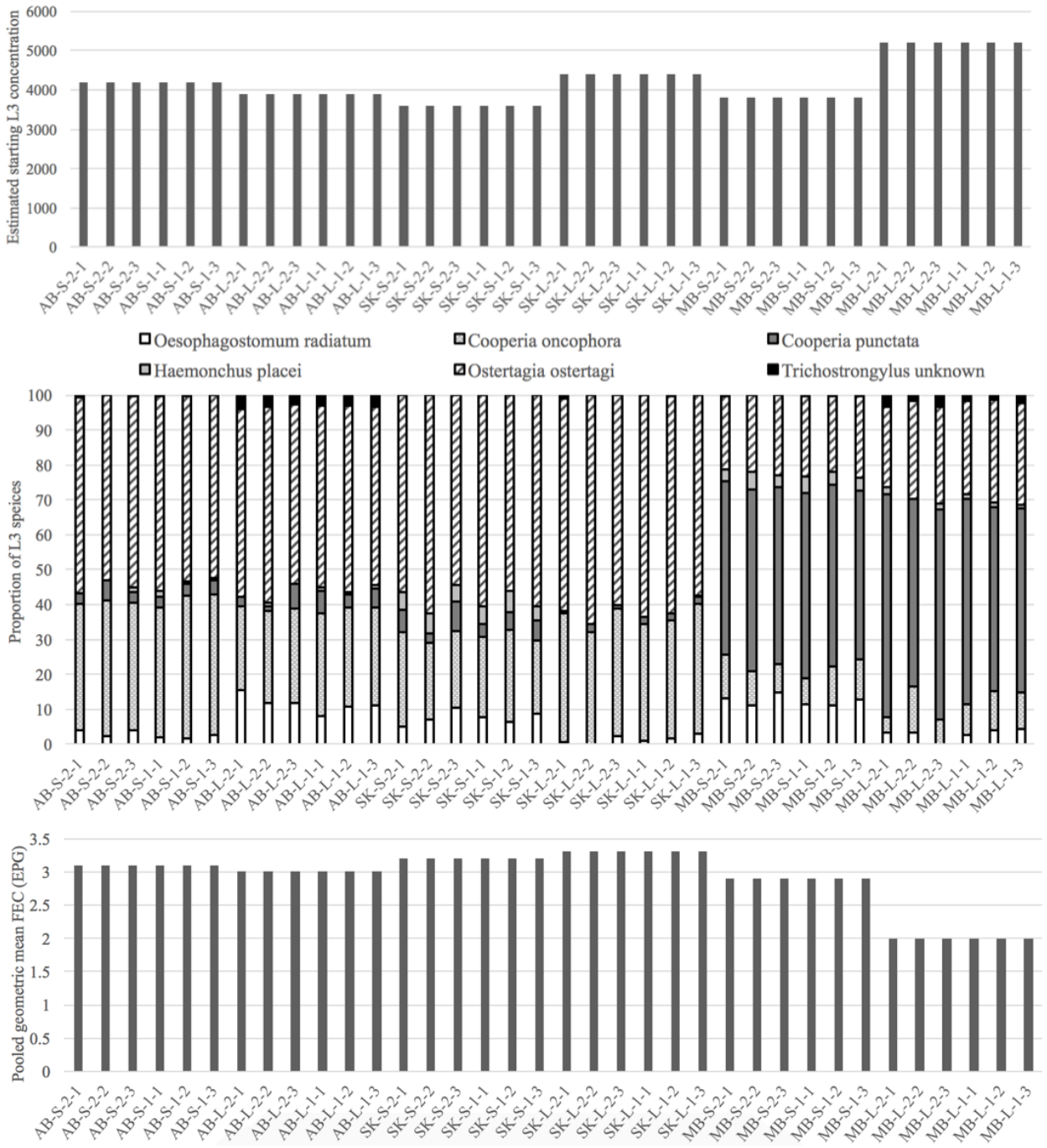

FIGURE 3.2 Describes (A) Geometric mean fecal egg counts (EPG; raw data). (B) Nematode species from deep-sequencing nemabiome assay of the ITS-2 rDNA region of L3 larvae. Bars represent 100% of the larvae in the sample. (C) Estimated starting number of L3 larvae for each pooled sample. All samples (n=36) denoted by province, herd size (S≤300, L>300) and L3 larvae (2=200L3, 1=1000L3) concentration and replicate number.

CHAPTER FOUR

SURVEY OF GASTROINTESTINAL NEMATODE MANAGEMENT IN COW-CALF HERDS FROM THE PRAIRIE PROVINCES OF WESTERN CANADA

Felicity K. Wills[1]; John R. Campbell[1]; Sarah E. Parker[1]; Cheryl L. Waldner[1]; Fabienne D. Uehlinger[1].

[1]From the Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK, S7N 5B4, Canada.

This chapter represents the analysis of the current management of gastrointestinal nematode burdens in beef cattle from the western Canada, from samples collected from a pilot disease surveillance network – Western Canadian Cow-Calf Surveillance Network – aimed at providing a mechanism for the uniform and systematic collection of information from the western Canadian Cow-Calf industry, similar the National Animal Health Monitoring System in the United States. A questionnaire was created to gather information on animal and pasture management and the current use of parasite control products by these producers. There is a paucity of current information in the literature about the management of gastrointestinal nematodes in these herds. Research does however show that the management of these nematodes can have an impact on the development of anthelmintic resistance, which poses a threat to future control of the production limiting effect of these parasites. The results of this survey showed that the management of gastrointestinal nematodes in western Canada with parasite control products is almost entirely restricted to the use of macrocyclic lactone drugs as a pour on application and dosed based on a visual estimation of the animal's weight. Routine treatment in the fall and a lack of monitoring of parasite burden to guide the need for treatment or determining treatment efficacy were also very common. All these factors have been associated with the development of anthelmintic resistance.

It is important to understand current management strategies so that more sustainable management advice can be development for these herds, that limits the development of anthelmintic resistance.

AUTHOR CONTRIBUTIONS: Uehlinger, Campbell, Waldner and Parker were responsible for helping to design the questionnaire, data analysis and manuscript review. Wills was responsible for of the development of the questionnaire and anthelmintic control products handbook, design and analysis of data and manuscript preparation.

4.1 Abstract

Beef cow-calf production in the three prairie provinces of Alberta (AB), Saskatchewan (SK) and Manitoba (MB), which encompass almost over 70% of all cow-calf beef production in Canada, represent an important sector of the Canadian agrarian economy. All grazing cattle are exposed to gastrointestinal nematodes (GIN) and the GIN burdens are a cause of lost productivity in grazing herds. There is a paucity of information from western Canadian beef cow-calf producers about how they manage GIN. The objectives of this study here were: 1) to describe the current cow-calf pasture and cattle management practices as they may relate to GIN burden; 2) to describe current parasite control product usage; and 3) to define cow-calf producers' opinions and sources of information on GIN management. A questionnaire was developed and distributed to 105 producers in the Western Canadian Cow-Calf Surveillance Network (WCCCSN), a pilot disease surveillance network in May 2016. The responses from 97 of these producers revealed the almost uniform dependence on the use of a pour-on macrocyclic lactone parasite control product in the fall as part of a routine farm management program, as the method of choice for the treatment of GIN in western Canadian beef cow-calf herds. The choice of methods for controlling GIN in these herds raise the question of their impact on the development of anthelmintic resistance. There is no current information on the level of anthelmintic resistance in western Canada and this would be a direction for future research.

4.2 INTRODUCTION

Beef cow-calf production in the three prairie provinces of Alberta (AB), Saskatchewan (SK) and Manitoba (MB), which encompass almost over 70% of all cow-calf beef production in Canada, represent an important sector of the Canadian agrarian economy (Statistics Canada, 2017). Cost efficient beef production is important socioeconomically, particularly with the demand to feed a growing population (Sanders, 2007). All grazing cattle are exposed to gastrointestinal nematodes (GIN) and the GIN burden is a major cause of lost productivity in grazing herds (Lawrence and Ibarburu, 2006; Stromberg and Gasbarre, 2006).

It can be very difficult to quantify the economic costs of GIN burden in beef cow-calf herds, because of the difficulties in quantifying the subclinical impact on production of these burdens. Stromberg & Gasbarre (2006) and Grisi et al. (2014) examined the economic losses caused by GIN in cattle in the US and Brazil respectively and found them to be between two and seven billion dollars annually. A meta-analysis of 170 research trials by Lawrence et al. (2006) suggest that the economic benefit of GIN management to the cattle industry is 2.5 times greater than the use of growth promotors. There is no current information on the cost to production of GIN in western Canada, however, given some of the similarities in FEC intensities compared to those seen in the northern US states, the cost maybe comparable (Gilleard, 2016).

At present, the majority of livestock producers administer anthelmintic treatments without supporting diagnostic or epidemiological evidence (Kenyon and Jackson, 2012). 'Blanket' and routine anthelmintic drug treatment strategies provide producers with improved production rates; however, such approaches place intense selection pressure on parasite populations, resulting in a reduction in the population of parasites not exposed to anthelmintic drugs (Kenyon et al., 2009). That population of unexposed parasites is referred to as 'refugia'. A reduced 'refugia' population has been linked to the development of anthelmintic resistance (AR) (van Wyk, 2001).

In addition to routine 'blanket' treatment of stock with a parasite control product as a main mode of GIN management, other choices made by producers are likely responsible for the growing reports of AR in different GIN species (Gasbarre et al. 2009). A survey conducted by the National Animal Health Monitoring System (NAHMS) on beef cow-calf herds in the United States asked about several management strategies employed by producers, including choice of anthelmintic drug (AD) class, route of administration, method of dose calculation and timing of routine treatments (USDA, 2010). The results of that study found that the majority of producers

used a pour-on macrocyclic lactone (ML) product, administered it at a scheduled time in the herd's management program, and based the dose on visual estimation of average animal weight (McArthur and Reinemeyer, 2014). With beef producers' choices seemingly restricted to a single chemical class and a strong preference for convenient routes of administration, complacent adoption of scheduled programs and potential under dosing of animals, perfect conditions for the development of AR are created (Gasbarre 2001).

Pasture management also plays an important role in exposure of cattle to infective third stage GIN larvae (L3). The overarching aim of pasture management, in terms of GIN control, is to reduce pasture contamination with L3, i.e. to produce 'safe' pastures, and to reduce animal exposure to heavily contaminated pastures (Ballweber, 2006). This can be accomplished through deliberate stock and pasture manipulation. Periods of adverse weather conditions, very cold or very hot weather, can lead to death of infective larvae on pasture, resulting in 'safe' pastures (Yazwinski and Tucker, 2006). Several methods utilized for the creation of 'safe' pastures include spelling/resting pastures, alternating grazing different species and grazing newly sewn pastures/hay aftermath/crop stubble (Ballweber, 2006; Smith et al., 2009; Stromberg and Averbeck, 1999). Another method of pasture management is the use of rotational grazing systems. These systems involve the frequent movement of cattle (usually at higher stocking densities) through a number of pastures or sections of pastures, utilizing most of the available forage in order to stimulate regrowth.

There is a paucity of information from western Canadian beef cow-calf producers about their current opinions on GIN in their herds or how they manage GIN. Increasing reports of AR highlight the need to develop treatment strategies that incorporate contemporary animal and pasture management practices, in addition to chemical deworming, into the control of GIN burdens (Gasbarre 2014; Gasbarre et al. 2009; Gasbarre et al. 2009b). This is also important because of the increasing number of 'organic' producers who cannot rely on anthelmintic drugs for GIN control. However, to recommend economical and practical alternative anthelmintic control practices it is important to understand the current animal and pasture management strategies and producer opinions on GIN burdens (Morgan et al., 2013). Other countries including the United States have developed integrated information gathering systems, such as the NAHMS, which allows for the uniform and systematic gathering of data for timely comparisons in animal health and management factors between years and geographical regions (Animal Health

Australia, 2016; USDA, 2010). A similar monitoring network was recently established in western Canada with cow-calf producers. The Western Canadian Cow Calf Surveillance Network (WCCCSN) is comprised of approximately 120 beef cow-calf herds. Enrolled producers committed to participate in a six-year program that was set up to collect information on herd demographics, management systems and disease surveillance.

Within the context of the WCCCSN, the objectives of this study here were: 1) to describe the current cow-calf pasture and cattle management practices as they may relate to GIN burden; and 2) to define cow-calf producers' opinions towards and sources of information on GIN management. A questionnaire was developed and distributed to producers in the WCCCSN.

4.3 Materials and methods

4.3.1 Survey population

The recruitment process of producers into the WCCCSN has been previously described (Moggy et al., 2017; Waldner et al., 2017). Briefly, recruitment of producers into the WCCCSN was based on the last Canadian agricultural census and was aimed at creating a geographically representative sample of cow-calf enterprises in the three prairie provinces (Alberta, Saskatchewan and Manitoba) (Statistics Canada, 2011). Producers were recruited through their regular herd veterinarians. Recruitment was contingent on a willingness to participate in questionnaires, biological sample collection and a minimum herd size of 70 cows. At the time of distribution of this questionnaire there were 105 herds enrolled in the WCCCSN (52 in Alberta, 34 in Saskatchewan and 19 in Manitoba)

Producers enrolled in the WCCCSN in May 2016 were invited to participate in the parasite management questionnaire. Based on information provided at the time of recruitment in the WCCCSN, questionnaires were administered through both mail hardcopy and web formats. A reminder, including a hardcopy of the questionnaire, was sent to producers who had not yet returned their questionnaire in August 2016.

4.3.2 Questionnaire design

The questionnaire consisted of 22 questions and was divided into two parts comprising short answer, multiple-choice questions, and rating questions. The first section asked producers

to describe their herd demographics and their grazing and pasture management of cow-calf pairs and replacement heifers during the spring/summer grazing period of 2015. These questions focused on gathering information about the stocking density and the general method of stock/pasture management (e.g. rotation, continuous, intensive or a combination of these) and included questions about water sources. Questions regarding stocking density and pasture management were asked specifically about the first two months of the spring/summer grazing season, as this is the period in which the potential for significant pasture contamination with L3 is expected to be greatest (Ranjan et al., 1992; Stromberg and Gasbarre, 2006).

The second section of the questionnaire focused on the herds' current GIN management practices, including the use of parasite control products and the producers' opinions about GIN, and their information sources for GIN management. The survey was pre-tested with seven cow-calf producers from Saskatchewan who were not enrolled in the surveillance network. An illustrated handbook of parasite control products registered for use in beef cattle in Canada was supplied to aid producers in answering some of the questions. Appendix A4.1 provides a copy of the parasite management questionnaire distributed to participating producers. Appendix A4.2 provides a copy of the Parasite Control Product Handbook that was distributed to the participating producers.

4.3.3 DATA ANALYSES

All responses were entered into a commercial database (Excel 2011; Microsoft Corp., Redmond, Washington, USA) and imported into a statistical software package (StataSE version 14, Stata, College Station, Texas, USA).

Descriptive statistics were performed for each of the survey questions and depicted as frequencies, proportions (95% confidence interval (CI)), and mean (±SD; normally distributed variable) or median (interquartile range (IQR); non-normally distributed variable). Herd size was calculated based on the maximum number of cow-calf pairs reported by each producer for the spring/summer grazing period of 2015. The effect of herd size was examined by categorizing herds into those with 300 head or less (≤ 300 head) and those with greater than 300 head (> 300 head).

4.4 RESULTS

4.4.1 DESCRIPTION OF SURVEY RESPONDENTS

The response rate to the questionnaire was 93% (97/105). There were 51% (49/97) of respondents from AB, 35% (34/97) from SK and 14% (14/97) from MB. Responses to the survey were received from June 2016 to January 2017 with 73% (71/97) being received in June and July 2016. Herds with ≤ 300 head made up 69% (67/97) and herds with > 300 head 31% (30/97) of respondents. Table 4.1 describes the number of respondents to the survey by province and herd size.

4.4.2 BREEDING HERD MANAGEMENT DURING THE FIRST TWO MONTHS OF THE 2015 SPRING/SUMMER GRAZING PERIOD

The median number of cattle run by herds during the spring/summer grazing period was 197 (IQR 180) cow-calf pairs and ranged from 58-2,700, 40 (IQR 56) replacement heifers with a range of 0-575, and 4 (IQR 10) dry cows with a range of 0-84. Table 4.2 provides a summary of the median number and range of cattle reported in each province at the start of the 2015 spring/summer grazing period. The median number of breeding management groups utilized was 5 (IQR 5) and ranged from 0-18. Both cow-calf pairs and replacement heifers pastured together in breeding management groups in 92% (89/97; 95% CI 84-96) of herds. For the largest breeding management group reported by each herd for the first two months of the 2015 spring/summer grazing period, the median number of cow-calf pairs was 103 (IQR 115) and ranged from 24 to 975.

4.4.3 GRAZING MANAGEMENT CHARACTERISTICS

Respondents were asked to answer questions specifically for the first two months of the spring/summer grazing period of 2015. For 63% (59/94; 95% CI 52-72) of herds the spring/summer grazing period began in May and for 29% (27/94; 95% CI 20-39) it began in June. Figure 4.1 describes the percent (95% CI) of herds that begun the spring/summer grazing period by month and herd size. The median length of the spring/summer grazing period reported was 158 days (IQR 34) with a range of 87 to 246 days. The end of the spring/summer grazing period was reported as October by 54% (51/94; 95% CI 44-64) of herds and as November for

30% (28/94; 95% CI 21-40). Figure 4.2 describes the percent (95% CI) of herds that ended the spring/summer grazing period by month and herd size.

A rotational grazing system was the most commonly used system by all producers in both cow-calf pairs and replacement heifers. Of the respondents, 45% (44/97; 95% CI 35-55) utilized a rotational grazing system for cow-calf pairs and 50% (47/94; 95% CI 40-60) for replacement heifers. A combination of grazing systems (rotational and continuous) was used by 33% (32/97; 95% CI 24-43) for cow-calf pairs and by 11% (10/94; 95% CI 6-19) for replacement heifers. Figure 4.3 summarizes the grazing systems utilized by herds for both cow-calf pairs and replacement heifers by province. When herd size was examined, the most commonly used grazing system was rotational, for both herds with less than and greater then 300 head. A rotational grazing system was utilized by, 43% (29/67; 95% CI 30-54) of herds with ≤ 300 and 50% (15/30; 95% CI 34-65) of herds with > 300 head. For replacement heifers, 51% (33/65; 95% CI 39-63) of herds with ≤ 300 head and 48% (14/29; 95% CI 31- 66) of herds with > 300 head used a rotational grazing system.

Along with the type of grazing system utilized, producers were asked to describe the intensity of their stocking density for the largest proportion of their cow-calf pairs and replacement heifers. A light stocking density (<0.5 heifer or cow-calf pairs/acre) was utilized by 61% (58/95; 95% CI 51-71) of herds for cow-calf pairs and 61% (57/95; 95% CI 51-71) replacement heifers. A moderate stocking density (0.5-1 heifers or cow-calf pairs/acre) was reported by 28% (26/93; 95% CI 20-38) of herds for cow-calf pairs and by 31% of herds (30/95; 95% CI 23-42) for replacement heifers. Finally, 11% (11/95; 95% CI 6-19) of herds utilized an intensive stocking density (>1 heifer or cow-calf pair/acer) for cow-calf pairs and 8% (8/95; 95% CI 3.6-15.1) for replacement heifers. Figure 4.4 summarizes the stocking density utilized by herds for both cow-calf pairs and replacement heifers by province.

Of the respondents, 29% (28/97; 95% CI 21-39) utilized community pastures during the spring/summer grazing period of 2015. Of those herds that utilized a community pasture, the mean number of cow-calf pairs sent to community pasture was 36 (SD 68.2), the median number of cow-calf pairs was 0 (75^{th} percentile 60) and the maximum number of cow-calf pairs sent to community pasture was 300. While on community pasture, the median number of other herds that cow-calf pairs were exposed to was 0 (IQR 1) and the maximum number of herds they were

exposed to was 30. Thirty-seven percent (11/30; 95% CI 21-55) of herds with >300 head and 25% (17/67; 95% CI 16-37) of herds with ≤300 head utilized community pastures.

Producers were asked if their cows and replacement heifers had direct access to surface water (sloughs and/or dugouts). For cows, 98% (95/97; 95% CI 92-99) of producers indicated that their cows had direct access to surface water. For the herds that answered for replacement heifers, 84% (81/96; 95% CI 75-90) indicated that they had direct access to surface water. In herds with > 300 head, 87% (26/30; 95% CI 69-95) of cows and 83% (55/66; 95% CI 72-61) of replacement heifers had direct access to surface water.

4.5 Internal parasite control

4.5.1 Treatment with parasite control products

Between May 2015 and May 2016, 99% (96/97; 95% CI 93-100) of producers treated cows at least once with a registered parasite control product, while 49% (47/97; 95% CI 39-59) treated calves and 99% (96/97; 95% CI 93-100) treated replacement heifers at least once with a registered parasite control product. The median number of treatments with a parasite control product per year in cows and replacement heifers was 1 (IQR 0), and in calves it was 0 (IQR 1). Figure 4.5 depitics the number of treatments used per year with a parasite control product for cows, calves and replacement heifers for herds with ≤ 300 head and > 300 head, and for all production types. The number of treatments per year by herd size were similar. However, while 99% herds treated cows and replacment heifers at least once, only 49% of herds treated calves. Figure 4.6 describes the percent (95% CI) of herds that administered a parasite control product by month of the year to cows, calves and replacement heifers, for the last reported treatment. For treated cows, 45% (43/96; 95% CI 35-55) of producers applied the parasite control product in November and overall, the majority treated cows in the fall months (October to December). For the 44 herds that reported the date of treatment for calves, the pattern of most frequent application was split with 27% (12/44; 95% CI 16-43) treating in May and 25% (11/44; 95% CI 14-40) treating in November. Finally, for herds that reported the date of last treatment for the replacement heifers, 41% (39/95; 95% CI 11-27) of producers applied the parasite control product in November.

For herds that reported the method of application for each production type, the most

commonly used in all cases was a topical pour-on, with 96% (92/96; 95% CI 89-98) of herds utilizing a pour-on product in their cows, 82% (36/44; 95% I 70-91) of herds used a pour-on product in their calves, and 87% (83/95; 95% CI 79-93) of herds utilized a pour-on product in their replacement heifers. In feed or mineral mix was then next most frequent route of administration in all animal types. Three percent (3/96; 95% CI 0.9-9) of producers used an in feed/mineral mix in cows; all of these, however, were used in combination with a pour-on product. Fourteen percent (6/44; 95% CI 1-23) of herds used an in feed/mineral mix alone or in combination with a pour-on in their calves and 14% (12/95; 95% CI 2-15) of herds used an in feed/mineral mix alone or in combination with a pour-on in their replacement heifers. Other routes of administration were very infrequestly used but included an oral drench in cows by one producer and an injectable product in calves by 2 producers.

Two classes of parasite control products were utilized by herds: macrocyclic lactones (ML) and benzimidazoles (BZ) (or a combination of the two (ML/BZ)). Figure 4.7 describes the percent (95% CI) of herds that utilized each class of parasite control product, by herd size and animal production type. For all animal production types, the most commonly used class of parasite control product was a macrocyclic lactone with, 96% (92/96; 95% CI 89-98), 86% (38/44; 95% CI 72-94), and 87% (83/95; 95% CI 79-93) of cows, calves and replacement heifers, being treated respectively.

In cows, a combination of ML/BZ was the next most frequently used type of parasite control product, while in calves and replacement heifers, a benzimidazole was the next most frequently used drug. Overall, 17 herds treated their animals with a BZ product. There were nine herds that treated cows with a BZ product, six out of these herds also treated their calves and eight treated their replacement heifers with BZ. No other herds treated their calves with a BZ product; however, seven further herds utilized a BZ product in their replacement heifers. Sixteen of the herds that treated with a BZ product were from Alberta (n=8) and Saskatchewan (n=8) and the remaining herd was from Manitoba. Nine herds with ≤ 300 head and 8 herds with > 300 head treated with a BZ product.

When applying parasite control products, 74% (71/96; 95% CI 64-82) of producers utilized visual estimation of the animal's weight to calculate the dose required, while a further 15% (15/96; 95% CI 9-23) utilized a weigh scale. The remaining 11% (11/96; 95% CI 6-20) utilized other methods of calculating dose rate, including estimated averages of animal weights

based on historical records. When herd size was examined, herds with >300 head utilized a weigh scale for dose calculation more commonly than herds with ≤300 head (5/30 and 9/67, respectively).

4.5.2 Producer opinion on gastrointestinal nematode management

Producers were asked about their reasons for choosing to use a parasite control product. Of the 97 responses, 47% (95% CI 38-58) made the choice to treat because it was a routine herd management practice. A further 29% (95% CI 21-39) treated with a parasite control product in order to control external parasites and 10% (95% CI 6-18) indicated they were directed to by a veterinarian. Although that answer option was available, none of the producers chose to use a product specifically for the control of internal parasites. Figure 4.8 depicts producers' reasons for choosing to use a parasite control product by herd size.

Veterinarians were the main source of information regarding the choice of parasite control product for 66% (95% CI 56-75) of producers. Anti-parasitic drug product representatives were the main source of information for a further 20% (19/97; 95% CI 13-29) and the remaining 14% (14/97; 95% CI 9-23) reported personal experience or knowledge from literature such as cattleman's magazines as their primary sources of information.

Producers were also asked to indicate, on a scale ranging from 'very important' to 'important' to 'not important' how factors such as product price, effectiveness in treating internal parasites, effectiveness in treating external parasites and ease of application, influenced their choice of product. Price was 'important' to 68% (66/97; 95% C1 58-77) of producers, effectiveness in treating internal and external parasites was 'very important' for 61% (59/97; 95% CI 51-70) and 63% (61/97; 95% CI53-72), respectively, while ease of application was 'important' for 54% (52/97; 95% CI 43-63). Figure 4.9 depicts the percent of herds that classified the factors of price, efficacy for the treatment of external parasites, efficacy for the treatment of internal parasites and ease of application, as very important, important or not important in their choice of parasite control product, by herd size.

4.5.3 Gastrointestinal nematode fecal egg count monitoring

Lastly, producers were asked if fecal egg counts (FEC) had been used in the past 3 years

as a way of monitoring GIN infection in their cattle. If a FEC had been conducted, they were asked to indicate in which production type they were used. Of all respondents, 65% (63/97; 95% CI 55-74) indicated that they had not used FEC in the past 3 years to monitor GIN burdens while 3% (3/97; 95% CI 1-9) did not know whether a FEC had been conducted. Figure 4.10 depicts the percent of herds that had fecal samples collected for monitoring of GIN infection by herd size and province.

Of the 32% (31/97; 95% CI 23-42) of producers that did have FEC performed, 24% (23/97; 95% CI 16-33) sampled mature cows, 13% (13/97; 95% CI 8-22) sampled replacement heifers, 4% (4/97; 95% CI 2-11) sampled steers and 2% (2/97; 95% CI 0-8) sampled calves. No bulls were sampled by the producers who responded to the questionnaire.

4.6 Discussion

This chapter presents the results of a questionnaire completed by beef cow-calf producers from the western Canadian prairie provinces that were enrolled in the WCCCSN. Results of the questionnaire describe the GIN management and opinions of 97 producers who returned the survey. There is little recent information available about the current management of GIN on beef cow-calf herds in western Canada, so it was the primary aim of this questionnaire to describe the current grazing management and parasite control products utilized by these producers. It was also an aim to establish producers' source of information about the management of GIN in their herds.

The responses of the producers to the administered questionnaire showed that for both mature cattle and replacement heifers the most commonly used grazing system utilized was a rotational grazing system. The number of herds that utilized a rotational grazing system for the first two months of the spring/summer grazing period was not dependent on province or herd size. In terms of the management of GIN, it has been suggested by Myers (1988) that rotational grazing systems may result in increased GIN burdens when compared to continuous grazing systems it results in closer grazing to fecal pats and lower down the sward resulting in increases exposure to infective L3 larvae. However, this is dependent on many factors including pasture species, rate of pasture regeneration and stocking density (Stromberg and Gasbarre, 2006). Specifically, the risk of increasing GIN burdens in rotational grazing systems seems to be associated with high stocking densities. From this current questionnaire, while rotational grazing

systems were frequently utilized, this was paired with light stocking densities for most of the producers and production types thus potentially reducing the risk of increasing GIN burdens. It is important to note, however, that the specific definition of rotational grazing systems is difficult as there is a wide variation in methods and timing utilized. Although a definition in the context of this questionnaire was supplied, producers may still have based their answer on a subjective understanding of their system or may have had to choose one of the available answer options even if none reflected their grazing system entirely; therefore, its subsequent interpretation must be viewed with a certain degree of caution.

The most striking information to come from producers' responses to the questionnaire is the almost uniform dependence on the use of a pour-on macrocyclic lactone parasite control product in the fall as part of a routine management program. These preferences for treatment choice were not influenced by herd size. The results of this questionnaire agree with a pattern of treatment seen by Jelinski et al. (2016) who conducted a survey of GIN in 14 Saskatchewan beef herds over the summer of 2014 and found that all but three producers applied a macrocyclic lactone in the fall. A study conducted by Murray et al. (Murray et al., 2016) of 246 beef cow-calf producers largely from Alberta who found 91% of respondents treated cows with an antiparasitic in the fall, while only 69% treated calves. The application of a parasite control product late in the fall going into the winter confinement period is suboptimal for GIN, although it is a preferred option for the control of important ectoparasites such as lice that become important during the housing period (Jelinski et al., 2016; Stromberg and Averbeck, 1999). While treatment timing is appropriate for producers aim in controlling ectoparasites, it is not ideal for GIN management. This response indicates a need for more producer education about the importance of appropriate timing and better targeted parasite control treatments. In order to do this evidence of the economic benefits of targeted selective treatments in beef cow-calf herds is needed but which has not yet been established for western Canada.

Unlike for cows and replacement heifers where between 97 and 99% of herds treated at least once with a parasite control product, only 49% of herds treated calves, a similar pattern as seen by Murray et al. (Murray et al., 2016). For the treatments administered to calves there was more variation in the timing of treatment than was seen in cows or heifers. About half of treated calves received the treatment in the early spring. This would coincide with turnout to pasture. Similar to treatments applied to adult cows in fall, this is suboptimal timing for the control of

GIN based on known epidemiology in northern temperate environments and based on the expected low GIN burden in calves at this time of the year. Based on results of work done by Mackie (2016) on beef cows and calves in Ontario, the optimal time to treat calves with a parasite control product would be late June to early July. The movement of treatment of calves to this time, however, presents a logistical challenge for producers who rarely handle the herd in the period after turnout.

The results of this questionnaire are also similar to the results of a study into the management of GIN in cattle herds from the northern United States. This study involved the administrations of a questionnaire, and resulted in responses from 474 dairy and beef producers from 14 states in the northeastern United States. Similarly, in the 2007-2008 NAHMS study conducted on 2,872 beef herds in 24 US states, pour-on application of MLs were the preferred route and drug class used and application was based on routine farm schedule rather than the optimal treatment time for GIN (McArthur and Reinemeyer, 2014; USDA, 2010). Obviously creating one standard guideline for managing GIN in cow-calf herds is impossible because of large variations in locations and management programs of herds; however, some key consideration can be made to help reduce the risk AR development. Some of these guidelines include, reducing the risk of under dosing animals, using combinations or rotations of anthelmintic drug classes and ultimately monitoring the effectiveness of treatments applied (Gasbarre, 2014).

With recent reports of AR detected to both ML and BZ parasite control products in multiple species of GIN in the US, the management choices made by beef cow-calf producers must be carefully considered for their implications to the development of AR (Edmonds et al., 2010; Gasbarre, 2014; Gasbarre et al., 2009a, 2009b). Several of the management choices made by surveyed producers here have been associated with the development of AR including the potential under dosing of animals depending on dose calculation method (e.g. visual estimation vs. weigh scale), method of application and the blanket treatment of all animals in a herd (De Graef et al., 2013; Gasbarre, 2014; McArthur and Reinemeyer, 2014). The effectiveness of pour-on products has been questioned as they have been shown to result in under dosing because of variable uptake of drug influenced by weather, cleanliness and coat condition of the animals, accuracy of application and licking behaviour of the animals (De Graef et al., 2013). All of these practices contribute to placing increased selection pressure on the present GIN by reducing the

refugia population.

The results of this survey also highlight that while the use of parasite control products is highly prevalent and effectiveness of treatment against internal (and external) parasites was considered very important by most producers, monitoring of the effectiveness of treatment by producers was rare. Only 32% (31/97; 95% CI 23-42) of producers had a FEC performed in their cattle in the last three years. The use of FEC and fecal egg count reduction tests (FECRT) to monitor the effectiveness of treatment are important strategies to try to identify as early as possible the development of AR in herds (Sutherland and Leathwick, 2011).

While the recruitment of herds into the WCCCSN was directly aimed at creating a representative sample of beef cow-calf operation from the western Canadian prairie provinces, ultimately there is some degree of bias in the selection of these producers based on their motivations for participation in a longitudinal surveillance network. The response rate of 93% (97/105) is excellent for a questionnaire and non-response bias is unlikely to have significantly influenced the results here (Thrusfield, 2013). Recall bias may be another potential source of misinformation; the questionnaire asked producers to recall their herds' management for the previous (2015) grazing season and it is possible that not all producers completely remembered the requested information. So as with all voluntary response questionnaires there are some risks in applying the results to the wider population of beef cow-calf producers. However, the responses obtained here do represent a current source of information that may be used to direct future research in western Canada, including systematic evaluation of risk factors and assessing the levels of AR present in these herds.

4.7 Conclusion

The results of this questionnaire are a current summary of the management strategies utilized by beef cow-calf producers from the western Canadian prairie provinces, which was lacking from the literature. The responses are comparable to the NAHMS study in the US and highlight that the management of GIN in western Canada with parasite control products is almost entirely restricted to the use of macrocyclic lactone drugs as a pour on application and dosed based on a visual estimation of the animal's weight. Routine treatment in the fall and a lack of monitoring of parasite burden to guide the need for treatment or determining treatment efficacy

were also very common. All these factors have been associated with the development of AR in cattle GIN populations, and so despite a paucity of reported AR in western Canadian beef herds, some level of existing resistance would be expected. This is a clear knowledge gap in the current literature and future work needs to be conducted to establish the current level of AR in western Canadian beef cow-calf herds.

Another important point that comes from the responses to this questionnaire is the influence that the need to treat for ectoparasites has on the treatment of GIN in these herds. The effectiveness in treating ectoparasites was a stronger consideration to producers than the treatment of GIN. This needs to be considered in future management recommendations, both with regards to the timing of application and the choice of parasite control product.

4.8 TABLES

TABLE 4.1 Frequency and percentage of survey questionnaire responses by province and herd size.

	Herd size		
	≤ 300 head	> 300 head	All herds
Alberta	31/97 (32%)	18/97 (19%)	49/97 (51%)
Saskatchewan	24/97 (25%)	10/97 (10%)	34/97 (35%)
Manitoba	12/97 (12%)	2/97 (2%)	14/97 (14%)
Total	67/97 (69%)	30/97 (31%)	97/97 (100%)

TABLE 4.2 Descriptive summary of the number of each cattle production type reported by herds at the start of the 2015 spring/summer grazing season by province.

		Median	Interquartile range	Minimum	Maximum
Alberta					
	Cow-calf pairs	220	181	58	2,700
	Replacement heifers	40	56	10	440
	Dry cows	4	9	0	60
Saskatchewan					
	Cow-calf pairs	185	179	64	1,300
	Replacement heifers	42	57	0	575
	Dry cows	2	10	0	84
Manitoba					
	Cow-calf pairs	178	130	100	588
	Replacement heifers	34	37	0	109
	Dry cows	4	16	0	30
All herds					
	Cow-calf pairs	197	180	58	2,700
	Replacement heifers	40	56	0	575
	Dry cows	4	10	0	84

4.9 FIGURES

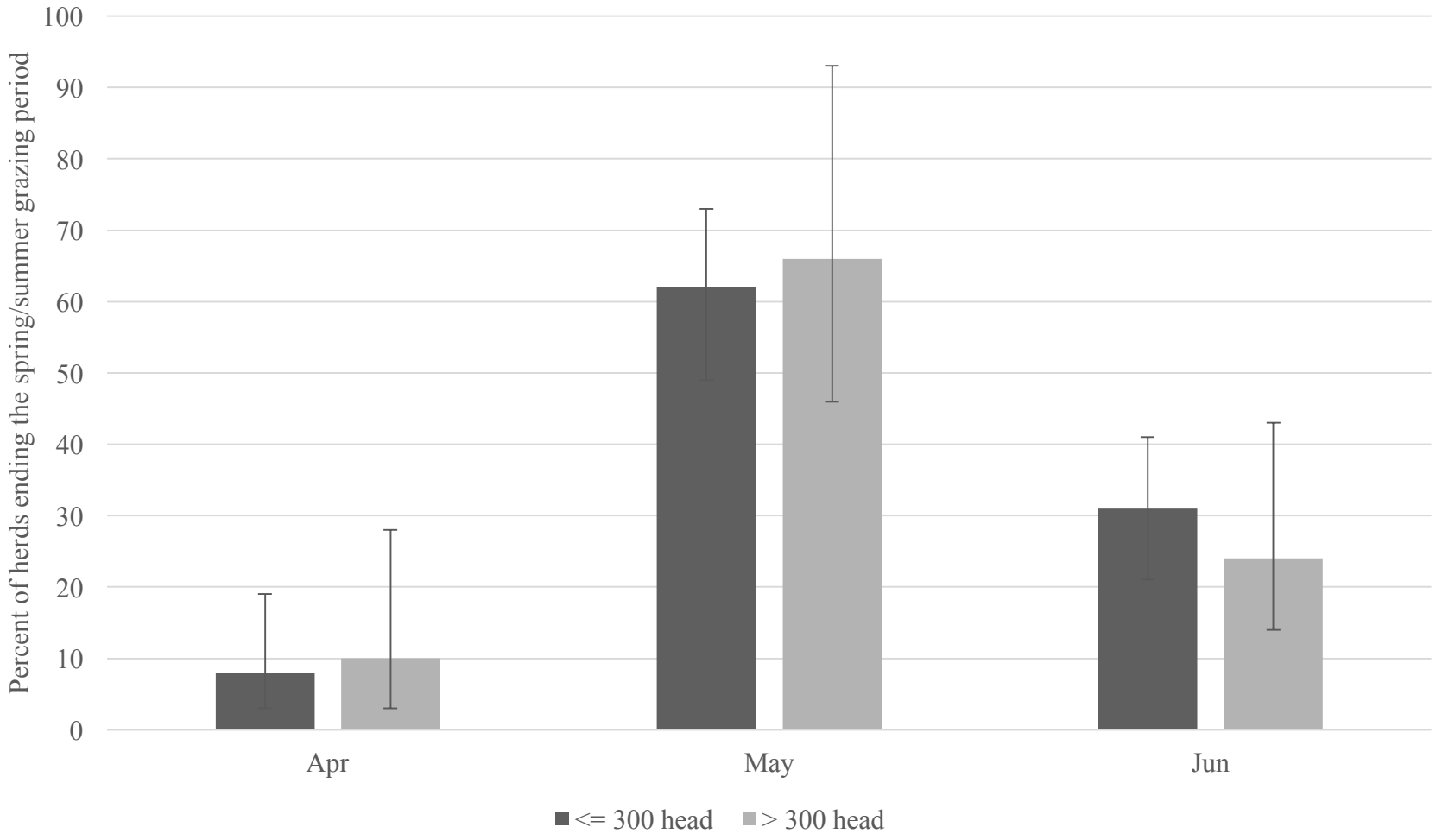

FIGURE 4.1 The percentage (95% CI) of herds (n=94) that started the spring/summer grazing season in April (Apr), May and June (Jun) in 2015, by herds ≤ 300 head (n=65) and herds > 300 head (n=29).

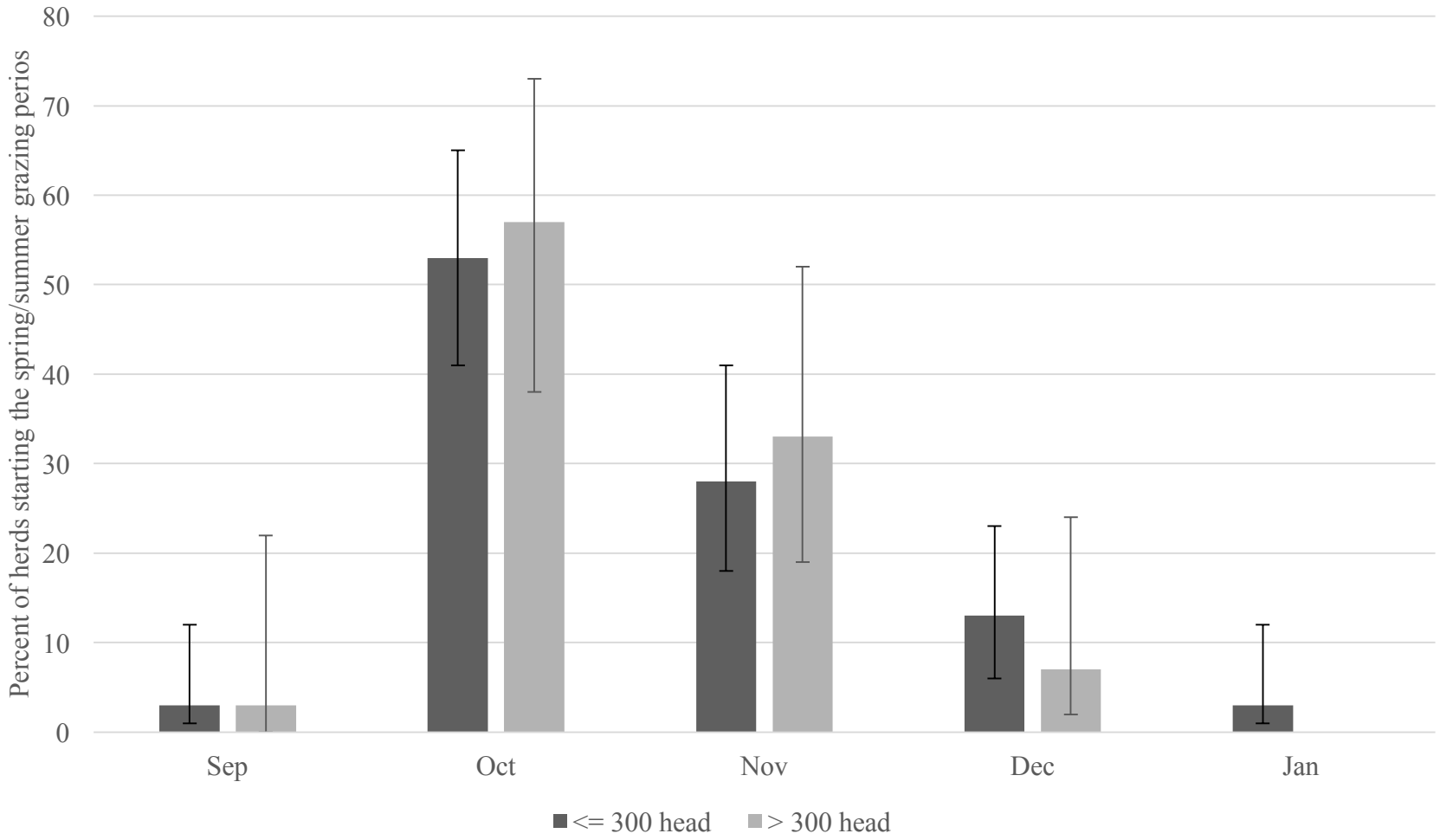

FIGURE 4.2 The percentage (95% CI) of herds (n= 94) that ended the spring/summer grazing season in September (Sep), October (Oct), November (Nov), December (Dec), January (Jan) and February (Feb) in 2015, by herds ≤ 300 head (n=64) and herds > 300 head (n=30).

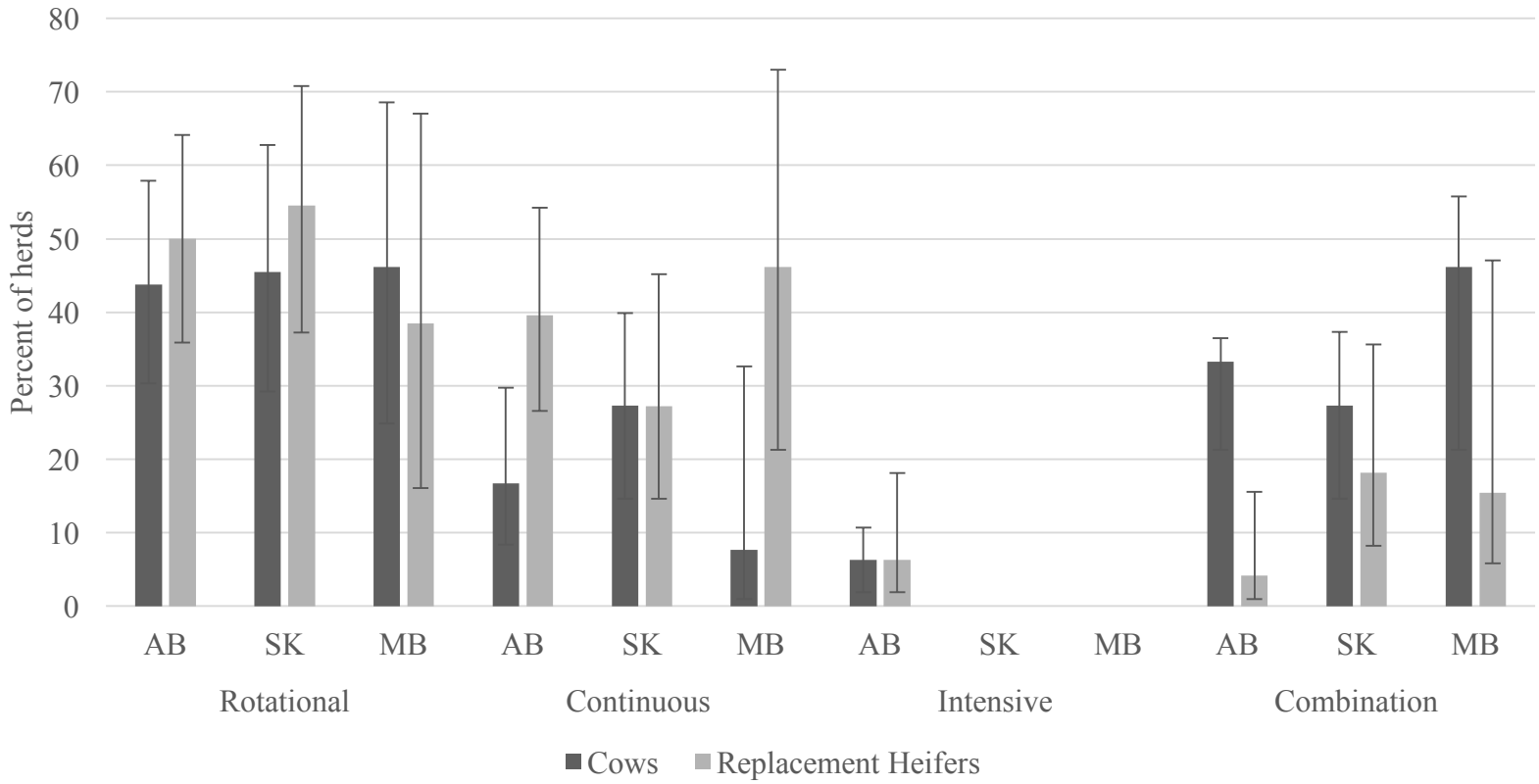

FIGURE 4.3 The percentage (95% CI) of grazing systems utilized by herds for cow-calf pairs (n=97) and replacement heifers (n=94) during the first two months of the spring/summer grazing period 2015, by province. *Continuous grazing* defined as cattle having free range and determine which areas of the entire pasture available to them they will graze. *Rotational grazing* defined as moving cattle through different pastures types but animal distribution is not directly managed (larger areas grazed for longer durations in rotation). *Intensive grazing* defined as the producer determining where, when and what livestock graze at a set stocking rate and directly control animal distribution and movement, utilizing small areas usually grazed for short durations (i.e. 1 week.) and in the same season going back onto the same pasture.

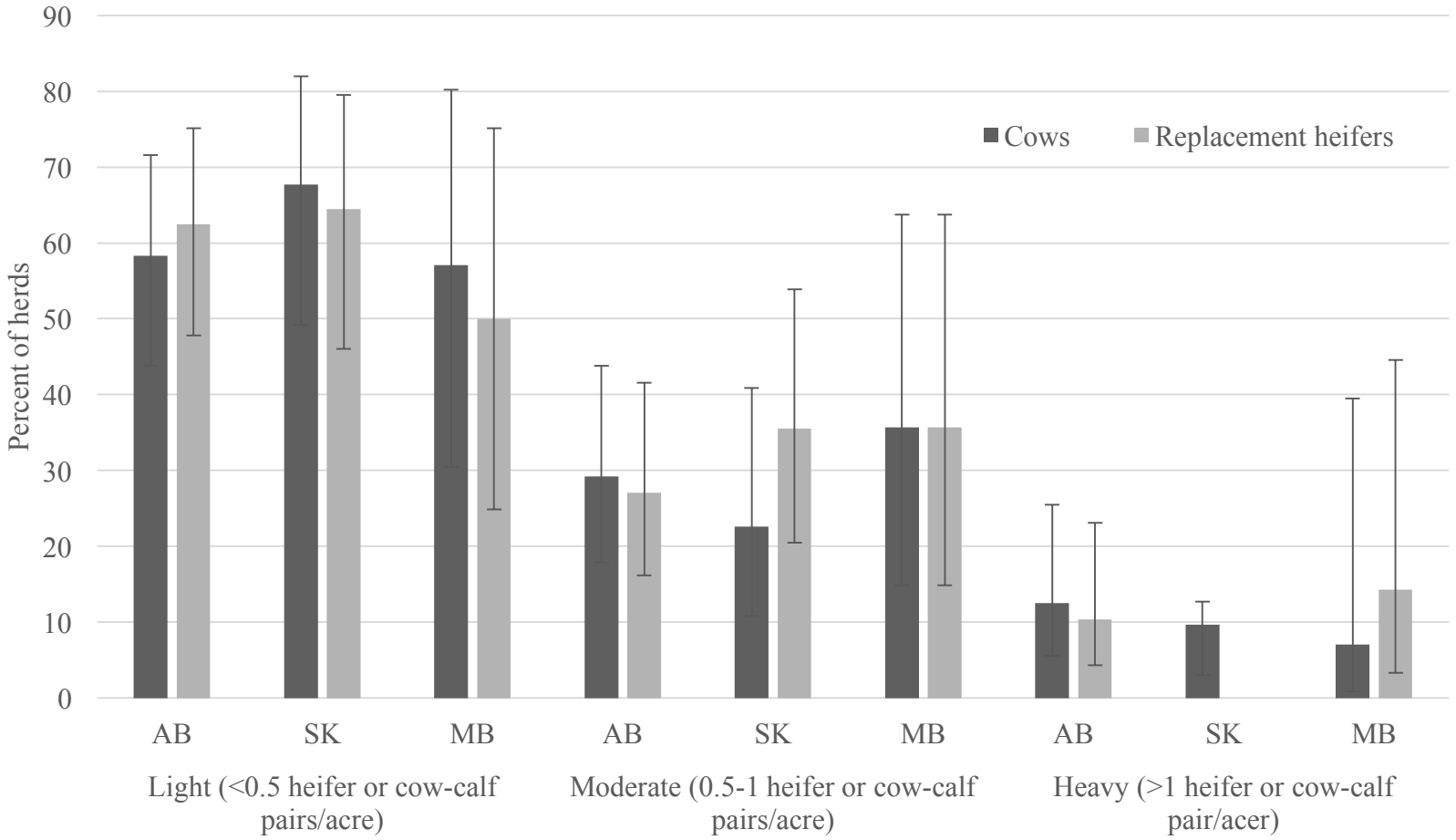

FIGURE 4.4 The percent (95% CI) of herds that describe the stocking density utilized by herds for cow-calf pairs (n=95) and replacement heifers (n=95) during the first two months of the spring/summer grazing period in 2015, by Alberta (AB), Saskatchewan (SK), Manitoba (MB).

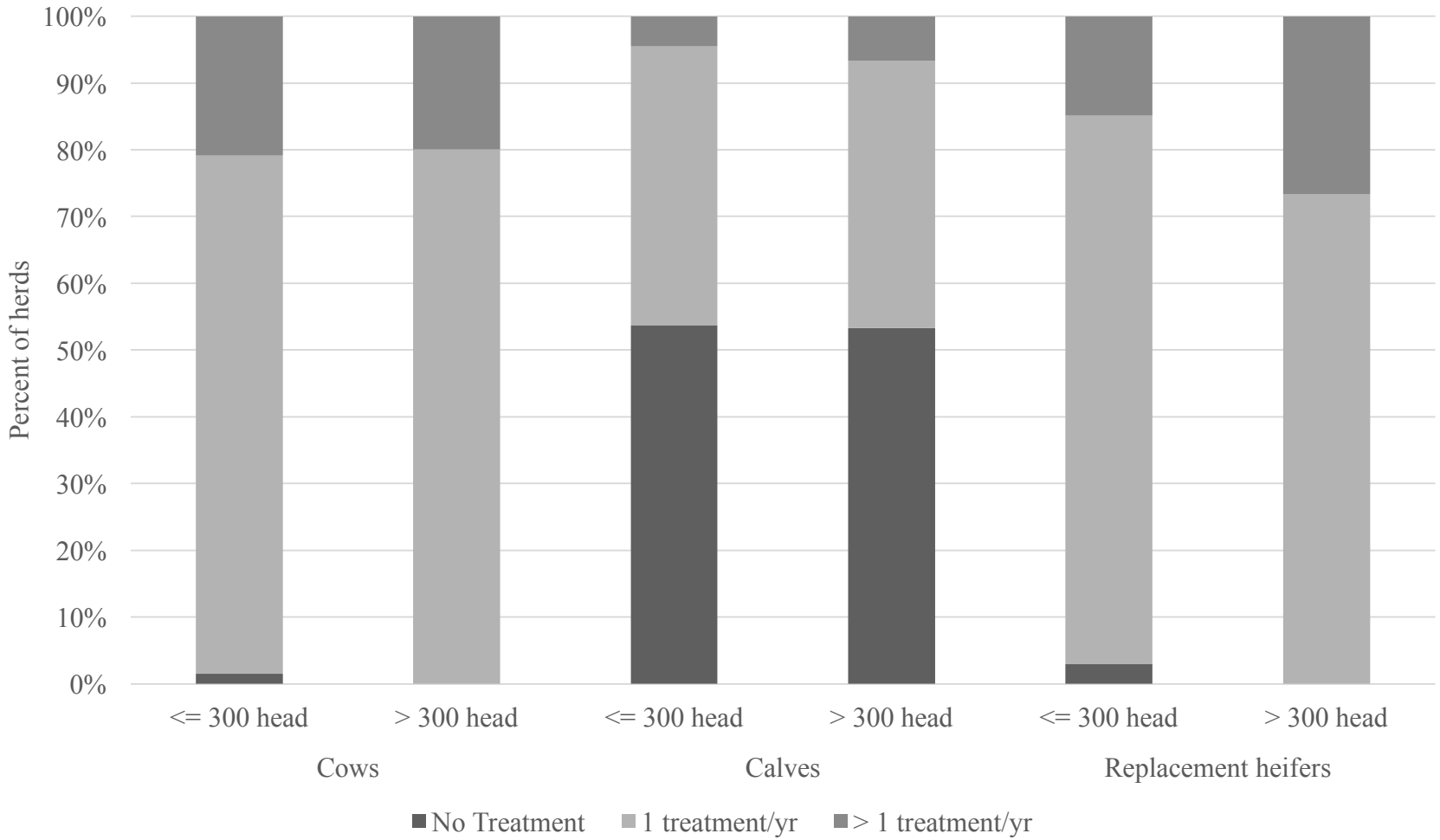

FIGURE 4.5 The percent of herds that treated cows (n=97), calves (n=97), replacement heifers (n=97) never, treated once per year or treated more then once per year, by herds ≤ 300 head (n=67) and herds > 300 head (n=30).

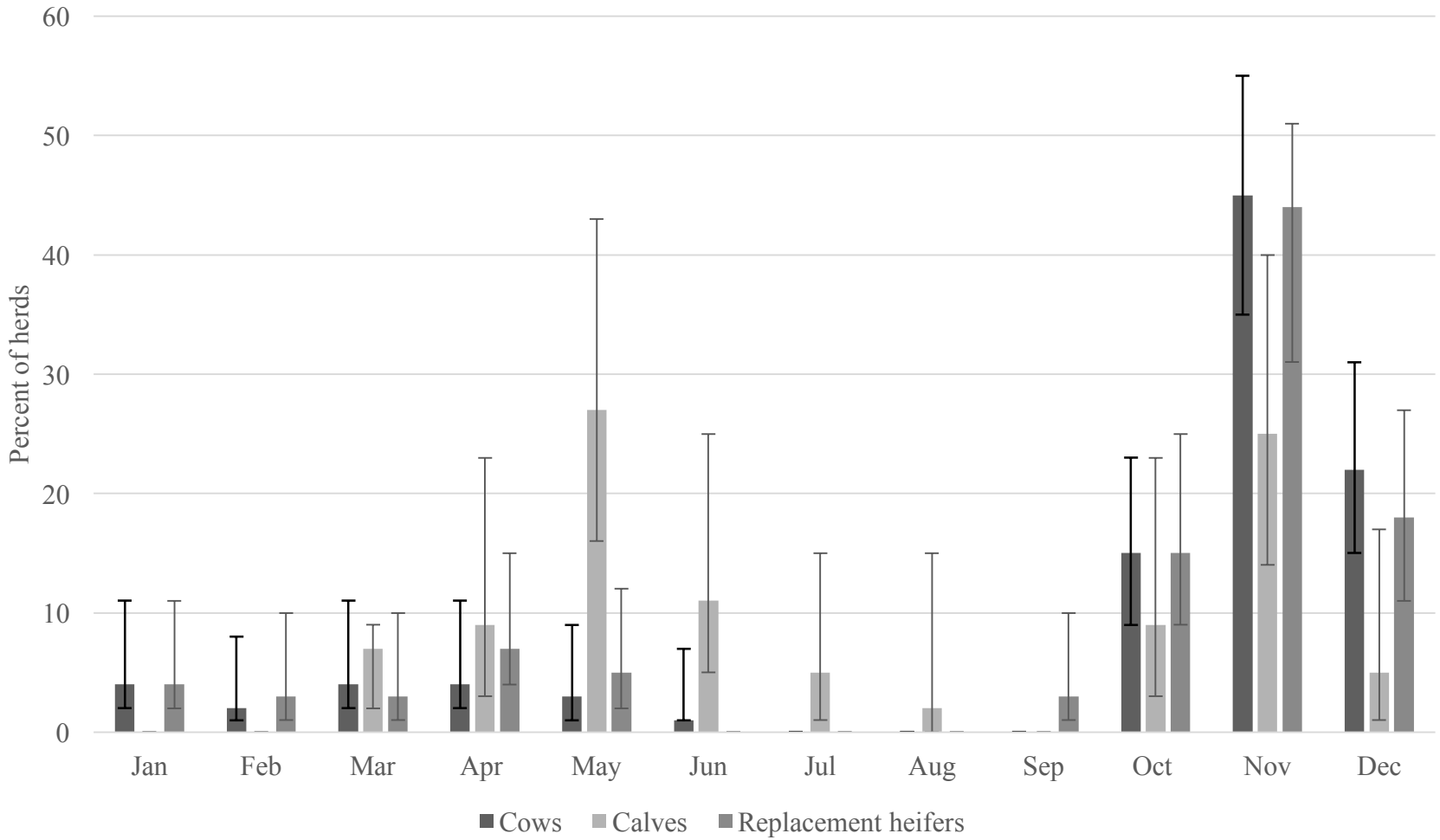

FIGURE 4.6 The percent of herds (95% CI) that administered a parasite control product by month of the year to cows (n=96), calves (n=44) and replacement heifers (n=95), for the last reported treatment.

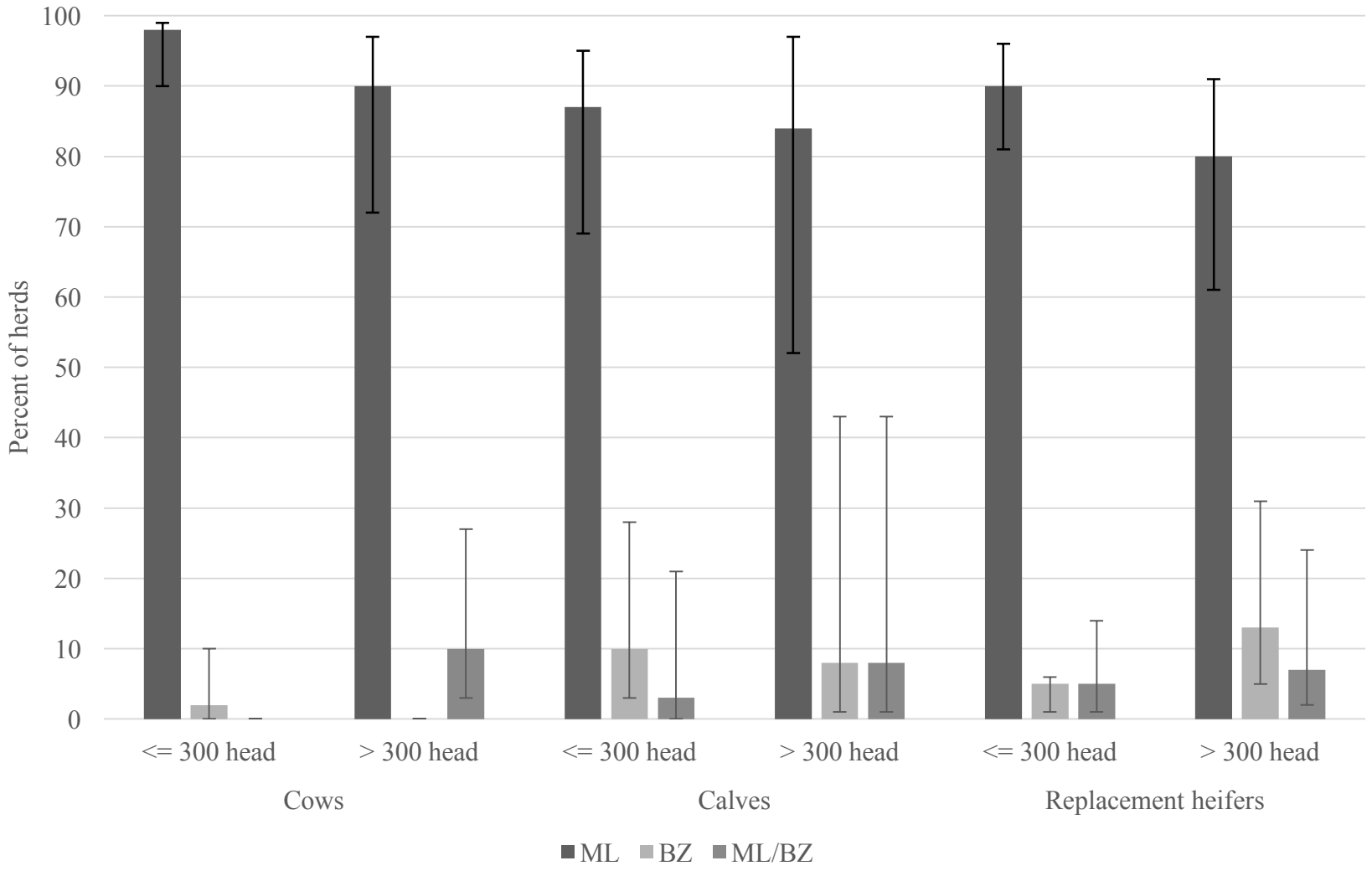

FIGURE 4.7 The percent (95% CI) of herds that treated cows (n=96), calves (n=44) and replacement heifers (n-95) with a macrocyclic lactone (ML), benzimidazole (BZ) or a combination of macrocyclic lactone and benzimidazole (ML/BZ) parasite control product, by herds ≤ 300 head (n=67) and herds > 300 head (n=30).

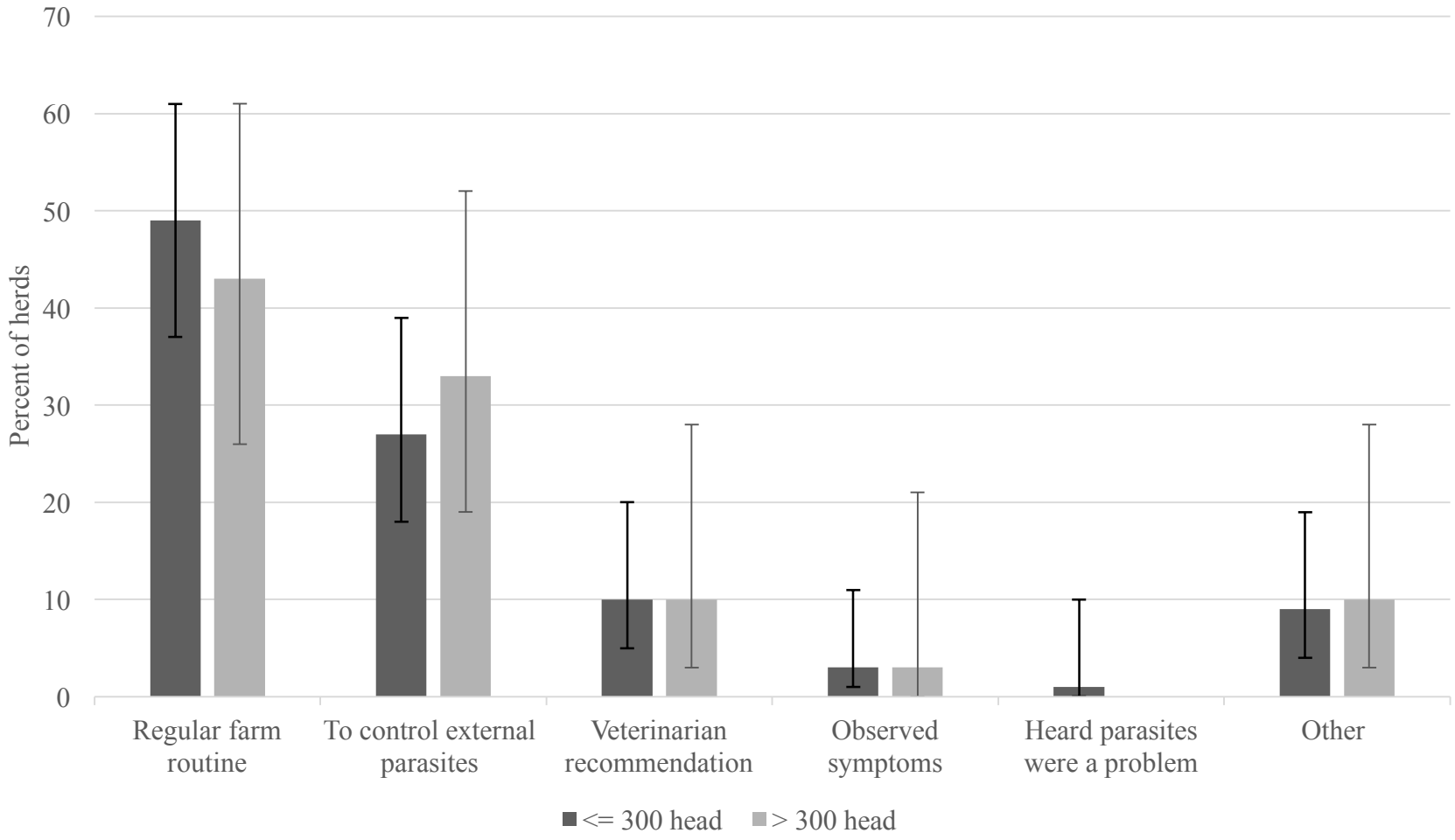

FIGURE 4.8 The percent (95% CI) of producers (n=97) that describe their reason to treat with a parasite control product, by herds ≤ 300 head (n=67) and herds > 300 head (n=30).

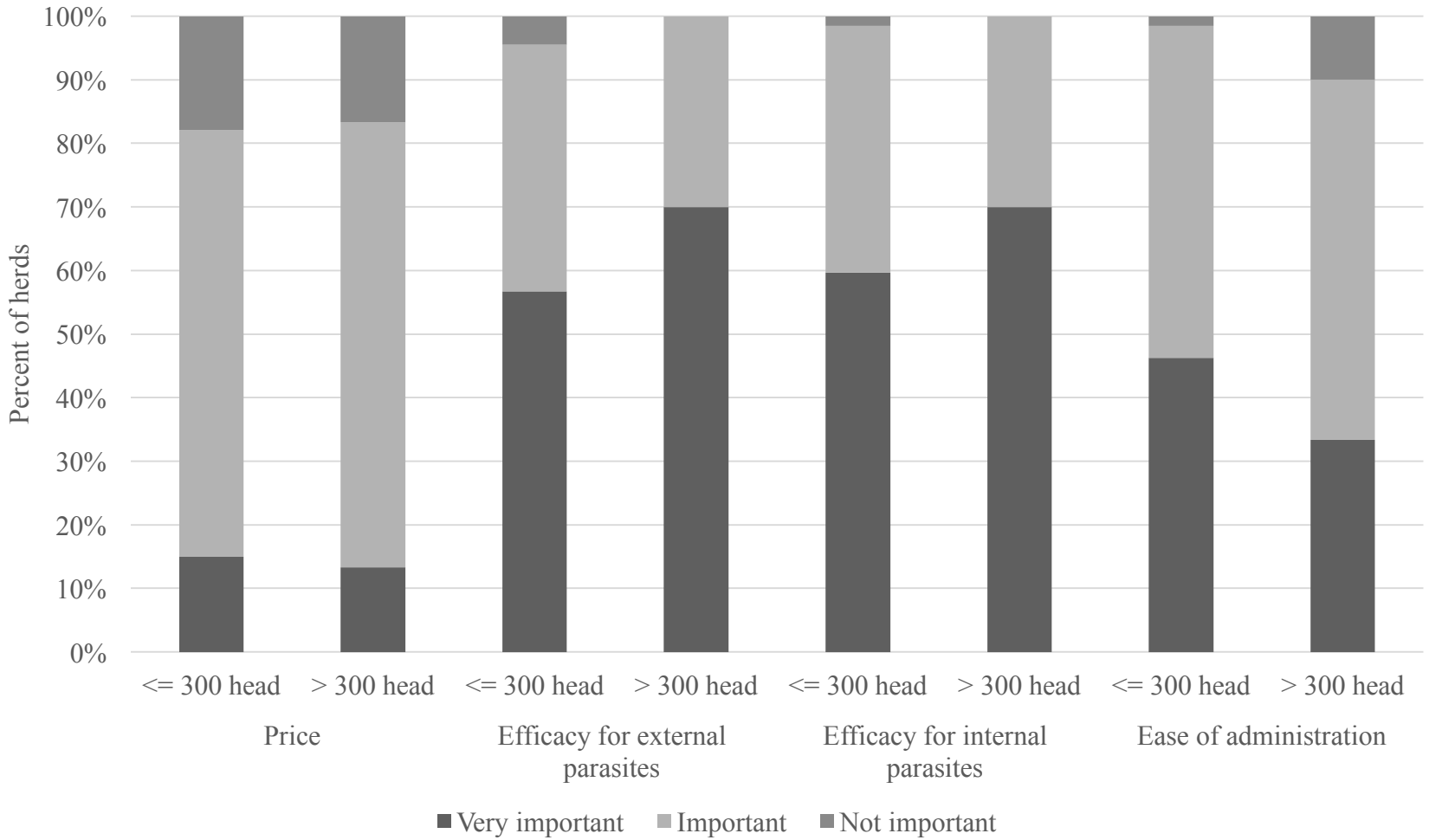

FIGURE 4.9 The percent of herds (n=97) that classified the factors of price, efficacy for the treatment of external parasites, efficacy for the treatment of internal parasites and ease of application, as very important, important or not important in their choice of parasite control product, by herds ≤ 300 head (n=67) and herds > 300 head (n=30).

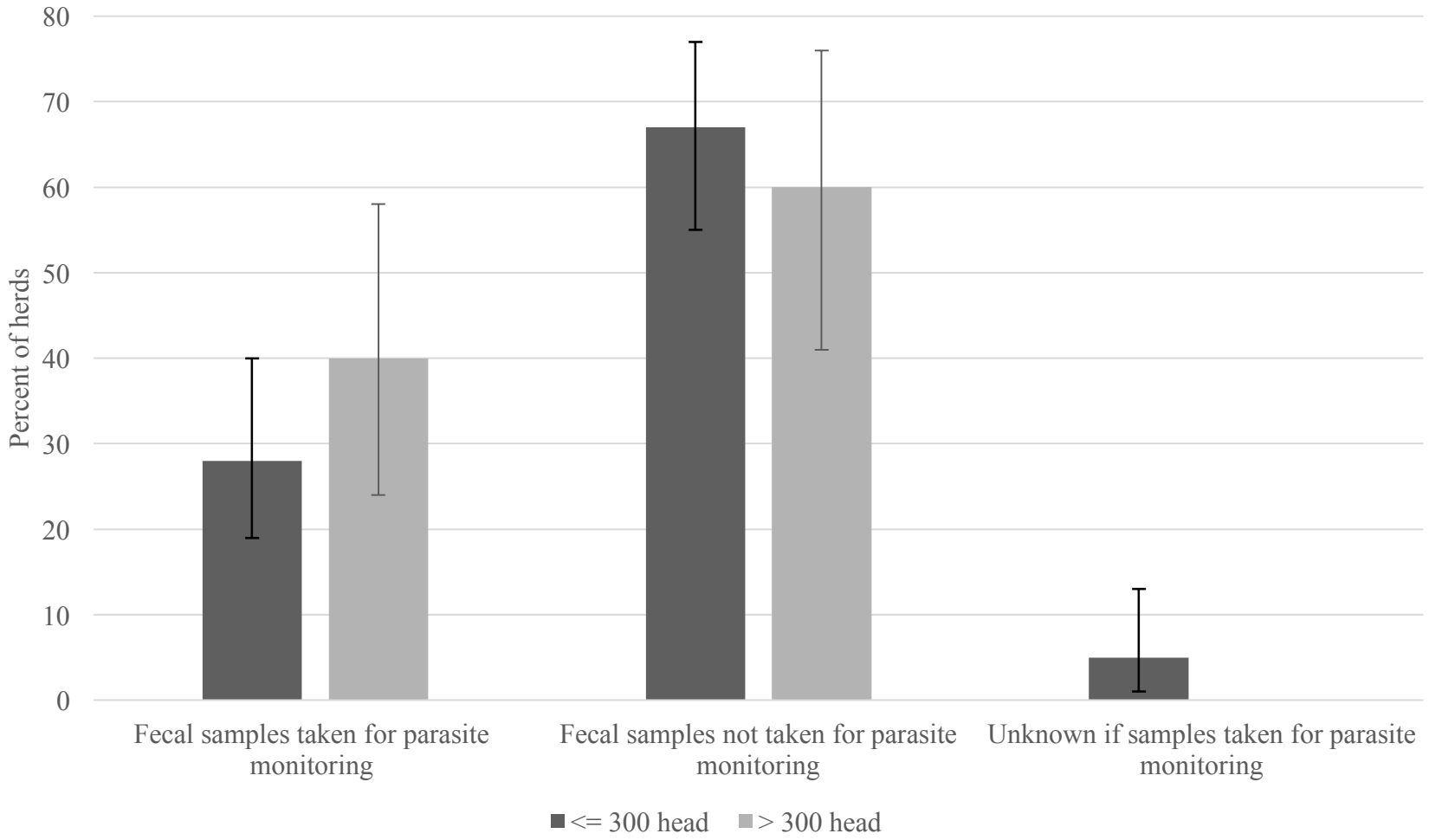

FIGURE 4.10 The percent of herds (n=97) that had fecal samples taken for parasite monitoring in the 3 years prior to the questionnaire date, by herds ≤ 300 head (n=67) and herds > 300 head (n=30).

CHAPTER FIVE

OBJECTIVE, DISCUSSION, GENERAL CONCLUSIONS AND FUTURE RESEARCH

5.1 OBJECTIVES

The overarching objectives of this thesis were to elucidate the epidemiology of gastrointestinal nematodes (GIN) in beef cow-calf herds of the western Canadian prairie provinces and to gain a better understanding of beef producers' management of and attitudes towards GIN in their cattle. In order to achieve these objectives, three separate studies (chapters) were conducted. Chapter 2 describes the GIN prevalence and FEC intensity in a convenience sample of beef cows, calves and replacement heifers in western Canada. Chapter 3 is focused specifically on herds enrolled in the Western Canadian Cow-Calf Surveillance Network (WCCCSN) and expands the knowledge gained in chapter 2 through more systematic sampling of beef heifers and analysis of prevalence and FEC intensity in the context of certain animal factors (age and body condition score). Furthermore, chapter 3 utilizing an emerging technique provides valuable information on species specific compositions of burdens, information that has been lacking to date. In both these chapters, a saturated sugar flotation and centrifugation/s technique was used to determine prevalence and FEC intensity while the predominant GIN species were identified using a next generation deep-sequencing assay of larvae harvested from coproculture in chapter 3. To address the third objective of this thesis, which was to elicit information on beef producers' current management practices and opinions on internal parasites, a questionnaire was developed, administered and results were summarized in chapter 4.

5.2 DISCUSSION

The information gained from the results of the three chapters in this thesis help to fill knowledge gaps in the current literature available on GIN in beef cow-calf herds in western Canada. Not since Bickis and Polley (1987) has there been a GIN prevalence study in western

Canada that has included multiple animal production types. More recent studies on the prevalence or burden of GIN in beef cow-calf herds of western Canada have focused on either one animal production type, a response to a control treatment or have been very specific to a geographic area (Beck et al., 2015; Jelinski et al., 2015; Schunicht et al., 2000). Knowing that GIN burdens vary so greatly between geographical regions and in fact from herd to herd (based on management factors and climate/environment) this makes it difficult to apply these results to a broader population such as the western Canadian prairie provinces.

The first two objectives of my thesis were to fill knowledge gaps in the current literature by providing current GIN prevalence and FEC intensity specific to beef cow-calf herds of the western Canadian prairie provinces. Chapter 2 was aimed at giving a very broad overview of the prevalence and FEC intensity that importantly encompassed multiple animal production types (cows, calves and replacement heifers). To do this, data collected by Merck Animal Health Canada during 2012, 2013 and 2014 was utilized. A saturated sugar and centrifugation technique was used to perform individual fecal egg counts (FEC) on samples submitted. This centrifugation technique has a minimum limit of detection of 0.5-3 EPG (Coles et al., 1992; Zajac and Conboy, 2012), which was considered advantageous, given that cattle frequently have low counts of eggs per gram of feces (EPG). The Trichostrongylid-type eggs, *Nematodirus* spp. and *Trichuris* spp. were differentiated morphologically but more in-depth analyses focused on Trichostrongylid-type egg prevalence and mean eggs per gram (EPG) of feces only because of the overall low prevalence of the other GIN. Perhaps not unexpectedly, the prevalence of Trichostrongylid-type egg positive samples was high in cows (76%), calves (81%) and replacement heifers (80%). When compared to literature from the northern US, this prevalence is comparable (85.6%) ((Stromberg et al., 2015). When compared to the study conducted by Bickis and Polley (1987) on cows (prevalence 53%) and calves (prevalence 65%) on community pastures and one intensively run herd (cows 63%), the prevalence found in this study were higher. However methodological differences such as the minimum detection limit of the FEC method used and freezing of the samples in the Bickis and Polley study make comparison difficult.

Fecal egg count intensity results from chapter 2 showed similar patterns to the prevalence results obtained. Calves had significantly (1.2 EPG) higher FEC intensity than cows (0.1 EPG) and replacement heifers (0.1 EPG). For both prevalence and FEC intensity, a significant interaction between season and production type was seen. This interaction revealed that cows

sampled in the fall had significantly lower prevalence and FEC intensity than calves or replacement heifers. This fits with the known epidemiology of GIN burdens in grazing beef cattle from temperate climates (Ranjan et al., 1992).

A limitation to this investigation was the lack of herd specific information, including exact geographical location or pasture management that could be used as predictors to draw more useful conclusions about risk factors that may influence the prevalence or burden of GIN. Another limitation of this analysis is the lack of GIN determination of larval species identity. It is known that cattle largely carry mixed burdens of GIN which differ in their epidemiology, fecundity and pathogenicity. This impacts on how the results of the FEC are interpreted and ultimately how management and/or treatment of these GIN should be undertaken (Tariq, 2015; Yazwinski and Tucker, 2006).

The second specific objective of this thesis was to characterize the herd-level gastrointestinal nematode burden of western Canadian cow-calf herds quantitatively (FEC) and qualitatively (determination of larval species identity), using a well-defined group of herds enrolled in the WCCCSN. Fecal samples were collected form 20 randomly selected heifers, from late September 2016 to February 2017 at fall pregnancy diagnosis. The number of fecal samples collected (n=20) from each herd was based on the guidelines of the World Association for the Advancement of Veterinary Parasitology and current literature (Gasbarre et al., 1996; Morrison, 2004; Wood et al., 1995). In total 1,655 samples were collected from 85 herds. The unadjusted predicted prevalence of Trichostrongylid-type egg positive samples was 92%. No significant differences were seen in the Trichostrongylid-type egg positive samples on the predictors identified. The level of FEC intensity was, however, consistently low with an unadjusted predicted mean EPG of Trichostrongylid-type eggs was 5.0.

Interestingly, herds with >300 cow-calf pairs had a significantly lower mean predicted Trichostrongylid-type EPG when compared to herds with ≤300 cow-calf pairs. The significant difference in the predicted mean EPG of Trichostrongylid-type eggs between different herd sizes brings up the potentially important discussion of different management practices utilized by these herds which may influence the level of burden. It could be speculated that large herds tend to be run more extensively than smaller herds, thus reducing animal exposure to infective L3 larva on pasture. This is particularly relevant to the current western Canadian beef cow-calf industry as the most recent Census of Agriculture completed in 2016 showed that while the number of herds was

decreasing the size of herds was increasing (Statistics Canada, 2017, 2016). It would be beneficial for these growing herds to be able to identify specific management strategies that would help to reduce the level of gastrointestinal nematode prevalence or burden.

Determination of larval species identity was also conducted on the samples used in chapter 3. There is no recent information published in beef cow-calves in western Canada on the predominant species compositions of GIN in these animals. In light of the importance (because of species specific differences in fecundity, pathogenicity and survivability) of knowing the species compositions in order to more accurately interpret the results of fecal egg count (FEC) diagnostics and make appropriate treatment choices, this information becomes very important to both producers and in particular veterinarians making treatment decision for these beef herds. Information about the predominant species is also important. Changes seen in the northern US, including increase in both *Cooperia punctata* and *Nematodirus* spp. particularly in calves, may have different effects on production and health. Both these species (*Nematodirus* spp. morphologically in chapter 2 and *Cooperia punctata* via deep sequencing in chapter 4) were seen in higher than expected numbers in the results of this thesis (Stromberg et al., 2015, 2012).

Filling the knowledge gap in understanding the current prevalence, FEC intensity, species composition and management of GIN in western Canadian beef cow-calf herds also becomes increasingly important because of the recent reports in the increase in anthelmintic resistance (AR) in cattle around the world, including our neighbors in the northern US (Edmonds et al., 2010; Gasbarre, 2014; Jackson et al., 2006; Sutherland and Leathwick, 2011; Waghorn et al., 2006). There is currently no published information on the state of anthelmintic resistance in beef cattle in western Canada, but it would be reasonable to assume that some level of anthelmintic resistance was present. Our ability to control GIN will rely on slowing the development of AR (Kaplan, 2004; Kaplan and Vidyashankar, 2012). In order to do this, current management strategies which, from the results of the producers' questionnaire from chapter 4, rely almost solely on the routine blanket application of a macrocyclic lactone pour-on product, will need to change to more target treatments (TT) or targeted selective treatment (TST) strategies (Kenyon and Jackson, 2012), in order to increase 'refugia' and decrease the selection pressure placed on GIN populations. In order for these strategies to be developed we need to have current information on the epidemiology and species compositions of GIN specific to western Canadian beef cow-calf herds, which this thesis starts to address.

A limitation in this study is the timing of sampling of heifers. The largest proportion of samples (64%) were collected in November and December 2016. Studies by Ranjan et al (1992) in Quebec found that FEC counts for grazing cattle peaked in May and early June and then began to decline into fall and winter. This decline in FEC is in some part attributed to the onset of hypobiosis in some GIN, like *Ostertagia ostertagi*, during the winter months. Therefore, sampling of these heifers during the fall was not ideal in identifying those peak levels of GIN burden and may have resulted in an underestimation of the FEC intensity. Ideally, these heifers and other animal production types would be sampled throughout the year in order to better document the epidemiology of GIN specific to beef cow-calf herds of the western Canadian prairie provinces.

These results attained from chapters 2 and 3, do however make biological sense. Calves having the lowest levels of immunity against GIN would be expected to have higher GIN burdens (Gasbarre, 1997). The seasonal effect seen in mature cows also fits with the known epidemiology of GIN in grazed cattle in temperature regions. As temperatures cool in the fall, the most common GIN seen in cattle including *Ostertagia ostertagi* undergo hypobiosis resulting in reduced egg output. The number of infective L3 on pasture also reduces because of less favorable environmental conditions for survival (Gibbs, 1988; Stromberg and Averbeck, 1999; Yazwinski and Tucker, 2006). The results of this investigation highlight that there are differences in the patterns of prevalence and FEC intensity between different production types and seasonally between production types, which should be considered when formulating management plans for these parasites and designing future studies into GIN in beef cattle in western Canada.

The uniformly high prevalence but low levels of GIN FEC intensity and limited differences seen across predictors such as age and body condition scores, raises the question of clinical significance of the results and being able to apply this information to a clinical setting or wider population. As far as the author is aware there has been no level of FEC or other parameter of GIN burden identified in grazed beef cattle, particularly mature cattle, that has been linked to economic and/or production measures that could direct more ‘targeted selective treatment’ of cattle. If available, this would potentially allow the beef producer to move away from the type of routine blanket treatment of cattle that was identified as the predominant management strategy in chapter 4 of this thesis.

The final objective of this thesis was to characterize the current management strategies employed by western Canadian cow-calf producers in the control of GIN, based on responses to a questionnaire administered to these producers. The questionnaire was developed and distributed to the 105 producers that were enrolled in the pilot disease surveillance network described above, at the time of administration. Responses were received from 97 of the 105 questionnaires administered, this is a response rate of 92%, which is an excellent response rate for a voluntary questionnaire, making the influence of certain types of bias (e.g. nonresponse bias) less of an issue in the study (Dillman et al., 2014). The responses from the producers revealed the almost uniform dependence on the use of a pour-on macrocyclic lactone parasite control product in the fall as part of a routine farm management program. The choice of methods for controlling GIN in these herds raise the question of their impact on the development of anthelmintic resistance. This is also important as management strategies employed by beef producers in this study have been linked to the development of anthelmintic resistance in multiple GIN to multiple classes of anthelmintic drugs. An increasing level of anthelmintic resistance could have serious impact for the beef cow-calf industry and its ability to control these production limiting parasites (Gasbarre et al., 2009b).

From the results of chapter 4 an important consideration that must also be noted is the use of anthelmintic drugs for the control of ectoparasites within these herds. From the questionnaire, the treatment of ectoparasites was an important consideration for producers in their treatment protocols, meaning when we start considering the development of targeted selective treatment programs for GIN, we must remember that producers will still have to consider the effective management of ectoparasites in their herds.

While the results of this survey have been very useful in identifying information about producers' management practices that have potential impacts on the development of AR in these herds, there is always some degree of inherent error when questionnaires are used to collect information about such complex biological systems. These errors are because of the complexity of some of the questions being asked with a lot of closed-ended and multiple-choice questions. These questions while they allow uniform capture of information may not reflect perfectly all the information as the 'preferred' or 'most correct' answer for each producer may not have been available. These issues along with issues such as recall bias, also mean that some degree of care must be taken when drawing conclusions from this data.

5.3 FUTURE RESEARCH

The effectiveness of parasite control programs utilizing 'blanket' routine treatment of cattle with anthelmintic drugs as the primary method of control over the last 3-4 decades, has resulted in limited research done into GIN in cattle. With current challenges facing the western Canadian beef cow-calf industry - including the need for more efficient beef production, climate changes and increasing reports of anthelmintic resistance from other parts of the world – it is important that more research is conducted into the current state of GIN in beef cow-calf herds in western Canada. Areas of future research that need to be addressed, include:

1) Defining further the species-specific epidemiology and risk factors of GIN in the different production types of beef cow-calf herds of the western Canadian prairie provinces and what impact they have on production.
2) Define the current level of species-specific anthelmintic resistance in GIN to the two most commonly used classes (macrocyclic lactones and benzimidazoles) of anthelmintic drugs in beef cow-calf herds of the western Canadian prairie provinces.
3) Define treatment thresholds in beef cattle, based on production indices, so that evidence based 'targeted selective treatment' strategies can be developed specific to beef cow-calf herds of the western Canadian prairie provinces.

One limitation of the research in this thesis that has been discussed above is the lack of longitudinal sampling in beef cow-calf herds with detailed herd geographical location and management information. This would be useful to more clearly elucidate the changing GIN prevalence and FEC intensity throughout the year, so that peak transmission times and risk factors can be better identified. Along with this the changing levels of GIN prevalence and burden in different animal classes need to be linked with the current impact that these burdens may have on production and/or reproduction in an attempt to identify treatment thresholds, similar to cut-off values being investigated in the dairy industry (Sekiya et al., 2013). This is particularly important as producers will require this information to be encouraged to adopt more 'targeted selective treatment' control programs. The use of the well-defined and described Western Canadian Cow-Calf Surveillance Network would be a perfect opportunity to do this, via the systematic sampling of cows, calves and replacement heifers, at multiple times throughout the

year and the use of the deep amplicon sequencing assay of the IST-2 rDNA locus to define species compositions.

Future studies investigating the current species composition in the different production types of grazed beef cattle of the western Canadian prairie provinces would be beneficial, as mentioned above. The results of this thesis showed some changes in the species composition of the GIN burdens, including higher prevalence on *Nematodirus* spp. and *Cooperia punctata*. *Nematodirus* spp., while not highly pathogenic has the ability to cause both clinical disease and reduced production in young naïve stock if not effectively controlled. *Cooperia punctata*, must be closely considered because the most frequent reports of anthelmintic resistance, particularly to ivermectin (a macrocyclic lactone) have been associated with *Cooperia* spp. Furthermore, *C. punctata* may also be more pathogenic than previously thought or than its relative, *C. oncophora*, which to date has been more commonly identified in grazing cattle. Along with the high levels of reported resistance in the *Cooperia* spp. there has also been research to suggest that the development of ivermectin resistance is linked to increased pathogenicity. Future research into this phenomenon would be beneficial as *C. punctate* appears to be on the rise (Wolstenholme et al., 2004).

Going hand in hand with studies into species compositions specific to animal production types, it would also be important to conduct studies to help determine the current level of species specific anthelmintic resistance present in western Canadian beef cow-calf herds. As mentioned above there is no current published information on the current state of anthelmintic resistance in beef cattle in western Canada. Trends worldwide, along with some preliminary work suggests that macrocyclic lactone pour-on often have below expected efficacy (Gilleard, 2016). With only two classes of anthelmintic drugs commonly use in western Canadian beef cow-calf herds (macrocyclic lactones and benzimidazoles) and no new products expected in the near future it is important that the continued development of anthelmintic resistance is slowed in order to preserve the efficacy of these drug. To do this, more emphasis needs to be placed on non-chemotherapeutic control methods and when required the use of evidence based targeted selective treatment programs.

The need for the development of evidence based targeted selective treatment raises the need for more quantitative diagnostic techniques, particularly for mature beef cattle. Fecal egg counts, which have historically been used for the diagnosis of GIN burdens in cattle, are poorly

correlated with actual adult worm burden once an animal has started to develop acquired immunity to GIN. Not being able to accurately quantify the level of GIN burden in individuals and in fact herds makes it very difficult to develop effective targeted selective treatment strategies. In the dairy industry, there have been some steps taken into the use of a commercial anti-*Ostertagia ostertagi* antibody ELISA to allow for the creation of quantitative treatment thresholds (Sekiya et al., 2013).

5.4 General conclusions

The results of this study provide needed epidemiological information about the prevalence, FEC intensity and species composition of GIN specific to western Canadian beef cow-calf herds, which is an important sector of the Canadian agrarian economy. It confirms that, as expected, the prevalence of GIN in grazed beef cattle is high. The findings support known epidemiological patterns for GIN transmission during the grazing season in temperate regions and the increased susceptibility of calves to GIN when compared to cows or replacement heifers. The risk factors leading to lower GIN burden in larger herds is noteworthy and warrants further investigations. This research also highlights that as has been seen in studies in the US there seems to be an increase in the levels of *C. punctata and Nematodirus* spp. in a mixed species burden, perhaps because of changes in the management of cattle and the climate, that warrants further investigation. Going forward, the high prevalence of GIN emphasizes the need for continuing research into this production limiting disease. Several areas of concern to be addressed would be more complete elucidation of GIN prevalence, intensity and species composition through multiple sampling time points and multiple animal production types from the same herds; elucidation of risk factors with a specific focus on small versus large herds; identification of species specific anthelmintic resistance, and the development of more accurate methods of diagnosing burden, particularly in adult beef cattle. In combination, this would further advance our knowledge and aid in developing evidence-based management strategies to reduce the risk of anthelmintic resistance development in western Canadian beef cow-calf herds

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APPENDIX A

TABLE A1.1 A summary of the outcome on production associated with use of some anthelmintic drugs on pastured cow-calf pairs, stocker calves and feedlot entrants, in climates where gastrointestinal nematode larvae are hypothesized or known to overwinter on pasture

Manuscript	Location	No. of herds	Animals	Interventions	Control group	Study length	Outcome on production
Pastured cows-calves							
(Forbes et al., 2002)	Southern England	4	334 late winter and spring-born suckled calves of both sexes, at least 3 months of age	Ivermectin sustained-release (SR) bolus	Untreated	3 years	Average rate of daily live weight gain was significantly higher in heifer and steer calves in the treated groups
(Kennedy et al., 1989)	Canada – Central Alberta	1	100 British-type steers (283-430kg)	2 treatments of Ivomec SC (200 μg/kg BW)	Untreated	120 days	Treated steers gained significantly more

							bodyweight over the 120 days of the trial then untreated steers
(Kennedy, 1990)	Canada - Alberta	2	178 Hereford cross cows and their calves 44 treated cows 44 treated calves 45 untreated cows 45 untreated calves	2 treatments of Ivomec SC (200 µg/kg BW)	Untreated	73 days	Significantly higher average daily weight gain in treated calves when compared to untreated calves

(Larson et al., 1995)	USA - KA	1	78 yearling beef heifers	2 treatments of Ivomec SC (200 µg/kg BW)	Untreated	210 days	Improved weight gain, earlier onset of puberty and improved pregnancy rate during a 60-d breeding season in treated heifers
(Stromberg et al., 1997)	USA - MN	2	72 cows/heifers and their calves	Cows- 2 treatments fenbendazole suspension orally at 5 mg kg-1 body weight Calves - fenbendazole suspension	Untreated	2 years	Improved average daily weight gain in treated calves. Improved pregnancy rate in treated cows.

				orally at 5 mg kg-1 body weight			
(Ciordia et al., 1987)	USA - GA	2	466 Hereford x Brangus cow-calf pairs	1 treatment of Ivermectin SC (200 µg/kg BW)	Untreated	120 days	Improved average daily weight gain in treated calves.
Stocker calves							
(Ballweber et al., 1997)	USA – WI, MS, AR, GA	4	48 – 108 weaned beef calves	Doromectin at 200 mcg/kg body weight SQ and Ivermectin at 200 mcg/kg body weight SQ and ivermectin at	No treatment	140 days	Increased average daily gain for each intervention over control calves

				500 mcg/Kg body weight topically			
(Cleale et al., 2004)	USA – AR, ID, IL & WI	4	150 steers/heifers per site	0.1 Long-acting injectable Moxidectin at 0.5 mL/50 kg body weight SQ	Excipients of 0.1 long-acting injectable Moxidectin at 0.5 mL/50 kg body weight SQ	56 days	Increased average daily gain for treated animals over control animals
(Epperson et al., 2001)	USA - SD	1	60 British cross spayed yearling heifers	Ivomec® SR bolus at 1 bolus/275–600 Lbs.	No treatment	162 days	Increased average daily gain for treated animals over

							control animals
(Kunkle et al., 2013)	USA – AR, ID, LA, MO, MN, OR & WI	7	475 Beef cross animals 133-335kg	0.05 Eprinomectin ERI at 1 mL/50 kg SQ	Excipients of Eprinomectin ERI at 1 mL/50 Kg SQ	120 days	Increase in body weight in treated calves over control calves
(Merts et al., 2005)	USA - SD	11 trials at 9 sites	799 Bos Taurus yearlings	Ivomec® SR bolus at 1 bolus/275–600 Lbs	No treatment	109–182 days	Increased average daily gain for treated animals over control animals
(Skogerboe et al., 2000)	USA – TN, LA & WI	3	108 male castrated beef calves	0.005 Doramectin pour-on solution at 500 mcg/kg	No treatment	140 days	Increased average daily gain for treated animals over

							control animals
Feedlot entrants							
(Bauck et al., 1989)	Alberta - Canada	1	6883 yearling mix breed steers entering a commercial feedlot	Topical ivermectin (0.5%) at 1.0mL/10kg bodyweight;	Topical fenthion (20%) at 12 mL/295 kg body weight	100 days on feed	Final weight gain, average daily gain and dry matter intake to gain ratio where improved in the ivermectin group as compared with the Fenthion group
(Jim et al., 1992)	Alberta – Canada	1	6,169 mix breed steers entering a	Topical ivermectin	Oral oxfendazole	120 days	Final weight gain, average daily gain,

			commercial feedlot		and fenthion topically		and the dry matter intake-to-gain ratio not were significantly improved for calves in the ivermectin group
(Schunicht et al., 2000)	USA - Nebraska	1	14,184 mix breed steers entering a commercial feedlot	Topical ivermectin	Oral fenbendazole and permethrin and fenthion topically	100 days	Final weight gain, average daily gain, and the dry matter intake-to-gain ratio were significantly improved for calves in the ivermectin group

TABLE A2.1 Raw prevalence (95% confidence interval) of Trichostrongylid-type nematodes in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014, by year and season of collection.

		Prevalence (95% CI)			
		Cows	Calves	Heifers	Overall total
2012					**84 (83-86) (1,584/1,880)**
	Spring/Summer	84 (81-86) (624/744)[a]	85 (82-88) (440/515)	87 (84-90) (349/400)	85 (83-87) (1,413/1,659)
	Fall	62 (52-70) (72/83)	97 (90-99) (74/76)	89 (71-97) (25/28)	77 (71-82) (218/221)
2013					**72 (70-75) (985/1,361)**
	Spring/Summer	75 (71-79) (358/478)	62 (54-69) (106/171)	72 (67-76) 303/423)	72 (69-74) (767/1,072)
	Fall	65 (54-75) (54/83)	78 (71-83) (125/161)	87 (73-94) (39/45)	75 (70-80) (218/289)
2014					**77 (73-80) (499/651)**
	Spring/Summer	82 (77-87) (196/237)	80 (71-87) (73/91)	91 (79-96) (49/54)	83 (79-86) (318/382)
	Fall	49 (40-58) (59/121)	84 (77-89) (115/137)	70 (36-91) (7/10)	68 (62-73) (181/268)
Total		76 (75-78) (1,363/1,780)	81 (79-83) (933/980)	80 (78-83) (772/960)	**79 (78-80) (3,068/3,891)**

[a] (number positive/number sampled)

TABLE A2.2 Raw descriptive summary of the Trichostrongylid-type eggs per gram of feces in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014, by year and season of collection.

		Cows			Calves			Heifers			Overall total		
		Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)
2012											**2.7 (4.1)**	**0-142.3**	**1.7 (5.3)**
	Spring/ Summer	2.6 (4.1)	0-142.3	1.7 (5.0)	3.1 (4.6)	0-80.7	2.0 (8.0)	2.0 (3.5)	0-63.0	1.3 (3.7)	2.6 (4.1)	0-142.3	1.7 (5.3)
	Fall	2.0 (3.3)	0-32.7	0.7 (3.0)	5.0 (3.1)	0-55.0	5.7 (7.8)	4.3 (4.6)	0-48.0	3.3 (14.2)	3.4 (3.6)	0-55.0	2.0 (7.0)
2013											**2.01 (3.5)**	**0-53.3**	**1.0 (3.3)**
	Spring/ Summer	2.0 (3.6)	0-42.0	1.0 (3.7)	1.6 (3.0)	0-19.3	0.7 (2.0)	2.2 (3.7)	0-53.3	1.0 (4.0)	2.02 (3.6)	0-53.3	1.0 (3.3)
	Fall	1.6 (3.1)	0-18.7	0.7 (2.0)	1.8 (2.9)	0-39.3	1.3 (2.3)	3.6 (3.1)	0-27.0	3.0 (7.0)	1.9 (3.1)	0-39.3	1.0 (2.7)
2014											**2.5 (4.3)**	**0-143.0**	**1.0 (4.7)**
	Spring/ Summer	3.4 (3.7)	0-43.4	3.0 (6.7)	2.2 (5.2)	0-143.0	0.7 (3.7)	1.7 (3.2)	0-15.7	1.67 (3.33)	2.8 (4.0)	0-143.0	2.0 (6.0)
	Fall	0.8 (2.5)	0-8.3	0.0 (0.7)	3.3 (5.1)	0-76.0	2.0 (8.3)	0.5 (1.6)	0-1.00	0.33 (0.67)	1.9 (4.7)	0-76.0	0.7 (2.8)
Total		2.3 (3.9)	0-142.3	1.2 (4.0)	3.0 (4.3)	0-143.0	1.7 (5.7)	2.0 (3.6)	0-63.0	1.3 (4.0)	**2.4 (3.9)**	**0-143.0**	**1.3 (4.3)**

TABLE A2.3 Raw prevalence (95% confidence interval) of *Nematodirus* spp. in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014, by year and season of collection.

		Prevalence (95% CI)			
		Cows	Calves	Heifers	Overall total
2012					**19 (17-20) (349/1,880)**
	Spring/Summer	4 (3-5) (28/744)[a]	44 (40-49) (228/515)	5 (3-7) (19/400)	17 (15-18) (275/1,659)
	Fall	23 (16-32) (27/117)	62 (50-72) (47/76)	-	33 (28-40) (74/221)
2013					**8 (6-9) (106/1,361)**
	Spring/Summer	1 (0-2) (3/478)	20 (15-27) (34/171)	4 (2-6) (16/423)	5 (4-6) (53/1,072)
	Fall	1 (0-8) (1/83)	25 (19-33) (41/161)	24 (14-39) (11/45)	18 (14-23) (53/289)
2014					**13 (11-16) (85/650)**
	Spring/Summer	2 (1-4) (4/237)	14 (8-23) (13/91)	13 (6-25) (7/54)	6 (4-9) (24/382)
	Fall	9 (5-16) (11/121)	36 (29-45) (50/137)	-	23 (18-28) (61/268)
Total		4 (3-5) (74/1,780)	36 (33-39) (413/1,151)	6 (4-7) (53/960)	**14 (13-15) (540/3,891)**

[a] (number positive/number sampled)

TABLE A2.4 Raw descriptive summary of the *Nematodirus* spp. eggs per gram of feces in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014, by year and season of collection.

		Cows			Calves			Heifers			Overall total		
		Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)
2012											**1.5 (3.3)**	**0-54.3**	**0 (0)**
	Spring/ Summer	1.7 (3.6)	0-21	0 (0)	1.2 (3.0)	0-18.7	0 (1)	1.4 (3.0)	0-17.6	0 (0)	1.3 (3.1)	0-21.0	0 (0)
	Fall	2.3 (4.0)	0-54.3	0 (0)	3.3 (2.8)	0-30.0	1.3 (4.5)	-	-	-	2.9 (3.3)	0-54.3	0 (1.3)
2013											**0.8 (2.9)**	**0-34.3**	**0 (0)**
	Spring/ Summer	0.8 (2.1)	0-1.3	0 (0)	0.5 (1.8)	0-2.3	0 (0)	0.5 (2.0)	0-1.6	0 (0)	0.5 (1.8)	0-2.3	0 (0)
	Fall	1 (0)	0-1	0 (0)	1.3 (4.0)	0-34.3	0 (0.3)	0.8 (1.9)	0-2.0	0 (0)	1.2 (3.6)	0-34.3	0 (0)
2014											**1.5 (3.5)**	**0-26.7**	**0 (0)**
	Spring/ Summer	0.3 (1.0)	0-0.3	0 (0)	1.2 (3.1)	0-5.0	0 (0)	0.5 (2.2)	0-2.3	0 (0)	0.7 (2.8)	0-5.0	0 (0)
	Fall	2.2 (1.9)	0-2.6	0 (0)	1.9 (3.8)	0-26.6	0 (0.7)	-	-	-	1.9 (3.4)	0-26.7	0 (0)
Total		1.8 (3.5)	0-54.3	0 (0)	1.4 (3.3)	0-34.3	0 (0.7)	0.8 (2.6)	0-17.6	0 (0)	**1.3 (3.3)**	**0-54.3**	**0 (0)**

TABLE A2.5 Raw prevalence (95% confidence interval) of *Trichuris* spp. in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014, by year and season of collection.

		Prevalence (95% CI)		
		Cows	Calves	Overall total
2012				**0.2 (0.1-0.6) (4/1,880)**
	Spring/Summer	0.1 (0.02-0.9) (1/744) [a]	0.2 (0.03-1.4) (1/515)	0.1 (0.03-0.5) (2/1,659)
	Fall	2.0 (0.4-7.0) (2/117)	0 (0/76)	0.9 (0.2-3.6) (2/221)
2014				**0.5 (0.1-1.4) (3/650)**
	Spring/Summer	0 (0/237)	0 (0/91)	0 (0/382)
	Fall	0 (0/121)	2.0 (1.0-7.0) (3/137)	1.1 (0.4-3.4) (3/268)
Total		0.3 (0.1-0.5) (3/1,780)	0.3(0.1-0.9) (4/1,151)	**0.2 (0.08-0.4) (7/3,891)**

[a](number positive/number sampled)

TABLE A2.6 Raw descriptive summary of the *Trichuris* spp. eggs per gram of feces in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014, by year and season of collection.

		Cows			Calves			Overall total		
		Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)	Geometric mean (SD)	Min-max	Median (IQR)
2012								0.3 (1)	0-0.3	0 (0)
	Spring/ summer	0	0	0	0.3 (1)	0-0.3	0 (0)	0.3	0-0.3	0 (0)
	Fall	0.3 (1)	0-0.3	0 (0)	0	0	0	0.3	0-0.3	0 (0)
2014								0.7 (3.9)	0-3.7	0 (0)
	Spring/ summer	0	0	0	0	0	0	0	0	0
	Fall	0	0	0	0.7 (3.9)	0-3.7	0 (0)	0.7 (3.9)	0-3.7	0 (0)
Total		0.3 (1)	0-0.3	0 (0)	0.7 (3.9)	0-3.7	0 (0)	0.5 (2.7)	0-0.3.7	0 (0)

TABLE A2.7 Final binomial GEE model with an exchangeable correlation structure, a logit link function and robust standard errors, for the predicted prevalence of Trichostrongylid-type egg positive fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014.

		Coefficient	Standard error	z value	p value	95% CI
Constant		1.81	0.19	9.53	0.00	1.44-2.18
Year						
	2012	Ref				
	2013	-0.76	0.23	-3.38	0.00	-1.2-(-0.32)
	2014	-0.31	0.29	-1.07	0.29	-0.88-0.26
Season						
	Spring/summer	Ref				
	Fall	-1.28	0.28	-4.50	0.00	-1.84-(-0.72)
Production type						
	Cows	Ref				
	Calves	-0.21	0.21	-0.96	0.34	-0.62-0.21
	Replacement heifers	-0.04	0.20	-0.20	0.84	-0.43-0.35
Season by Production type						
	Fall by Cows	Ref				
	Fall by Calves	1.70	0.37	4.59	0.00	0.97-2.43
	Fall by Replacement heifers	1.48	0.74	2.00	0.05	0.03-2.94

TABLE A2.8 Final negative binomial GEE model with an exchangeable correlation structure, a log link function and robust standard errors, for the predicted mean EPG of Trichostrongylid-type eggs in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014.

		Coefficient	Standard error	z value	p value	95% CI
Constant		2.92	0.22	13.13	0.00	2.49-3.36
Year						
	2012	Ref				
	2013	-0.50	0.24	-2.02	0.04	-0.97-(-0.02)
	2014	-0.31	0.27	-1.14	0.25	-0.84-0.22
Season						
	Spring/summer	Ref				
	Fall	-1.13	0.23	-4.93	0.00	-1.58-(-0.68)
Production type						
	Cows	Ref				
	Calves	0.17	0.41	0.42	0.68	-0.63-0.97
	Replacement heifers	-0.35	0.25	-1.44	0.15	-0.84-0.12
Season by Production type						
	Fall by Cows	Ref				
	Fall by Calves	1.16	0.32	3.66	0.00	0.54-1.79
	Fall by Replacement heifers	1.35	0.45	3.01	0.00	0.45-2.22
Year by Production type						
	2012 by Cows	Ref				
	2013 by Calves	-0.79	0.40	-1.96	0.05	-1.57-(-0.001)
	2013 by Replacement heifers	0.33	0.27	1.20	0.23	-0.21-0.86
	2014 by Calves	0.49	0.41	1.22	0.22	-0.30-1.29
	2014 by Replacement heifers	-0.43	0.52	-0.84	0.40	-1.45-0.58

TABLE A2.9 Final binomial GEE model with an exchangeable correlation structure, a logit link function and robust standard errors, for the predicted prevalence of *Nematodirus* spp. egg positive fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014.

		Coefficient	Standard error	z value	p value	95% CI
Constant		-3.05	0.40	-7.61	0.00	-3.84-(-2.27)
Year						
	2012	Ref				
	2013	-1.43	0.34	-4.24	0.00	-2.09-(-0.77)
	2014	-0.91	0.44	-2.07	0.04	-1.78-(-0.05)
Season						
	Spring/summer	Ref				
	Fall	0.87	0.37	2.33	0.02	0.14-1.60
Production type						
	Cows	Ref				
	Calves	2.88	0.40	7.22	0.00	2.10-3.66
	Replacement heifers	0.58	0.49	1.18	0.24	-0.38-1.54

TABLE A2.10 Final negative binomial GEE model with an exchangeable correlation structure, a log link and robust standard errors, for the predicted mean EPG of *Nematodirus* spp. eggs in fresh environmental fecal samples collected from 3,891 cows, calves and heifers from 201 herds from the western Canadian prairie provinces sampled between 2012 and 2014.

		Coefficient	Standard error	z value	p value	95% CI
Constant		-0.95	0.39	-2.46	0.01	-1.71-(-0.19)
Year						
	2012	Ref				
	2013	-2.16	0.38	-5.62	0.00	-2.92-(-1.41)
	2014	-1.33	0.42	-3.18	0.00	-2.16-(-0.51)
Season	Spring	Ref				
	Fall	1.92	0.34	5.71	0.00	1.26-2.57
Production type						
	Cows	Ref				
	Calves	2.16	0.33	6.56	0.00	1.51-2.80
	Replacement heifers	-0.01	0.49	-0.02	0.98	-0.98-0.96

TABLE A3.1 Final binomial GEE model with an exchangeable correlation structure, a logit link and robust standard errors, for the predicted prevalence of Trichostrongylid-type egg positive fecal samples from 1,655 heifers from 85 herds from the western Canadian prairie provinces collected in the fall of 2016.

		Predicted prevalence				
		Coefficient	Standard error	z value	p value	95% CI
Constant		3.10	0.52	6.01	0.00	2.09-4.11
Herd size						
	≤300 cow-calf pairs	Ref				
	>300 cow-calf pairs	-0.01	0.42	-0.02	0.98	-0.83-0.81
Age						
	12 to 23 months	Ref				
	24 to 36 months	-0.26	0.36	-0.72	0.47	-0.97-0.45
BCS						
	≤2.5	Ref				
	>2.5	0.05	0.39	0.13	0.90	-0.71-0.81
Month of submission						
	Sep/Oct	Ref				
	Nov/Dec	-0.79	0.39	-2.01	0.05	-1.6-(-0.02)
	Jan/Feb	-0.29	0.45	-0.64	0.52	-1.18-0.60

TABLE A3.2 Final negative binomial GEE model with an exchangeable correlation structure, a log link function and robust standard errors, for the predicted mean Trichostrongylid-type EPG in fecal samples 1,655 heifers from 85 herds from the western Canadian prairie provinces collected in the fall of 2016.

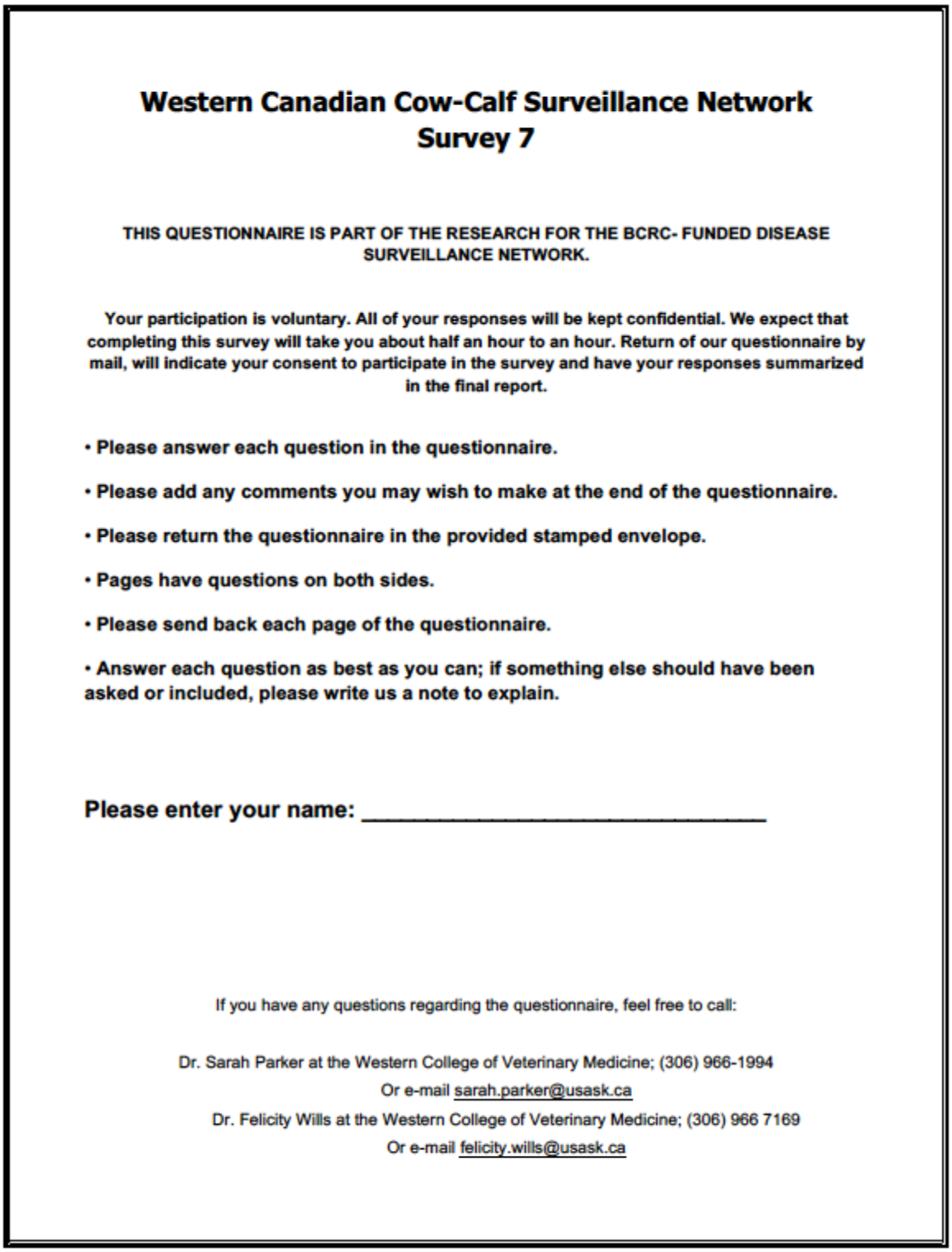
		Predicted mean EPG				
		Coefficient	Standard error	z value	p value	95% CI
Constant		3.32	0.14	23.49	0.00	3.04-3.59
Herd size						
	≤300 cow-calf pairs	Ref				
	>300 cow-calf pairs	-1.29	0.17	-7.75	0.00	-1.6-(-0.97)
Age						
	12 to 23 months	Ref				
	24 to 36 months	-0.18	0.25	-0.71	0.48	-0.67-0.32
BCS						
	≤2.5	Ref				
	>2.5	-0.07	0.09	-0.79	0.43	-0.25-0.11
Month of submission						
	Sep/Oct	Ref				
	Nov-Dec	0.21	0.16	1.32	0.19	-0.10-0.53
	Jan-Feb	0.26	0.25	1.04	0.30	-0.23-0.76
Herd size by Age						
	>300 by 24-36	1.01	0.29	3.49	0.00	0.44-1.58
Herd size by BCS						
	>300 by >2.5	1.30	0.25	5.16	0.00	0.80-1.79
Herd size by Age by BCS						
	24-36 by >2.5 by ≤300	-0.28	0.23	-1.21	0.23	-0.74-0.17
	24-36 by >2.5 by >300	-1.24	0.27	-4.68	0.00	-1.76-(-0.72)

TABLE A3.3 Description of the samples utilized for the deep amplicon sequencing assay for the ITS-2 rDNA locus used for determination of larval species identity of gastrointestinal nematode composition. For each province and herd size combinations a triplicate of samples with 200 L3 larvae per sample and a triplicate of samples with 1000L3 larvae per sample were used.

	# herds	# Total pooled samples	Estimated L3 concentration	Pooled geometric mean EPG (±SD*)
Alberta ≤300 cow-calf pairs	30	6	4200	3.1 (3.4)
Alberta >300 cow-calf pairs	15	6	3900	3.0 (3.3)
Saskatchewan ≤300 cow-calf pairs	18	6	3600	3.2 (3.1)
Saskatchewan >300 cow-calf pairs	11	6	4400	3.3 (3.7)
Manitoba ≤300 cow-calf pairs	11	6	3800	2.9 (3.8)
Manitoba >300 cow-calf pairs	5	6	5200	2.0 (3.5)

*Standard deviation

FIGURE A4.1 Western Canadian Cow-Calf Surveillance Network Parasite management questionnaire.



Western Canadian Cow-Calf Surveillance Network
Survey 7

THIS QUESTIONNAIRE IS PART OF THE RESEARCH FOR THE BCRC- FUNDED DISEASE SURVEILLANCE NETWORK.

Your participation is voluntary. All of your responses will be kept confidential. We expect that completing this survey will take you about half an hour to an hour. Return of our questionnaire by mail, will indicate your consent to participate in the survey and have your responses summarized in the final report.

- **Please answer each question in the questionnaire.**
- **Please add any comments you may wish to make at the end of the questionnaire.**
- **Please return the questionnaire in the provided stamped envelope.**
- **Pages have questions on both sides.**
- **Please send back each page of the questionnaire.**
- **Answer each question as best as you can; if something else should have been asked or included, please write us a note to explain.**

Please enter your name: _____

If you have any questions regarding the questionnaire, feel free to call:

Dr. Sarah Parker at the Western College of Veterinary Medicine; (306) 966-1994
Or e-mail sarah.parker@usask.ca
Dr. Felicity Wills at the Western College of Veterinary Medicine; (306) 966 7169
Or e-mail felicity.wills@usask.ca

Western Canadian Cow-Calf Surveillance Network
Survey 7

For the following questions please answer as accurately as possible for your whole cow-calf herd.

If you have multiple groups that are managed differently please answer the question for the management group with the largest number of cow-calf pairs.

Note: These pasture questions refer to last year (2015).

Section 1: Grazing system and Pasture management

[Spring/summer pasture-grazing would be grazing on grass pastures]

1. During 2015 when were **the start** and **the end** of your spring/summer pasture-grazing period for your cow-calf herd?

Please put a specific date if known (for example: 14th May 2015); if you do not know the specific date, please indicate the date/week that you think most closely represents the start date of your grazing period.

START DATE: _____ [please write the month in text]

END DATE: _____ [please write the month in text]

2. What was the largest number total of **cow-calf pairs** you had during the **first two months** of the spring/summer pasture-grazing period in 2015?

Number of cow-calf pairs: _____

3. What was the largest number total of **replacement heifers** you had during the **first two months** of the spring/summer pasture-grazing period in 2015?

(Replacement heifers = heifers from weaning to 2 years of age kept for breeding purposes.)

Number of replacement heifers: _____

4. What was the largest number total of **dry cows** you had during the **first two months** of the spring/summer pasture-grazing period in 2015?

Number of dry cows: _____

5. How many management/breeding groups did you have for cows (cow-calf pairs) and replacement heifers at the start of the spring/summer pasture-grazing period in 2015?

a. Number of management/breeding groups: _____

b. Are replacement heifers and mature cows run in separate groups? (Please circle)

Yes No

Please indicate the number of management groups for each:

Number of replacement heifer management/breeding groups: _____

Number of mature cow management/breeding groups: _____

6. How many **cow-calf pairs** were in your **largest** management/breeding group during the spring/summer pasture-grazing period?

Number of cow-calf pairs: _____

7. Summer grazing system 2015 for cows:

a. During the summer grazing period of 2015 which of the following grazing systems would **best** describe the grazing system for your **cow-calf pairs** for the spring/summer pasture-grazing period?

(*Different grazing systems are described here. Please check the grazing system that* ***best*** *characterizes your grazing management for this management group.*)

Continuous grazing **-** The cattle have free range and determine which areas of the entire pasture available to them they will graze.
Rotational grazing **-** You move cattle through different pastures types but animal distribution is not directly managed. This grazing system utilizes larger areas, grazed for longer durations in rotation.
Intensive grazing **-** You determine where, when and what livestock graze at a set stocking rate and directly control animal distribution and movement, utilizing small areas usually grazed for short durations (i.e. 1 week.) and in the same season going back onto the same pasture.

☐ Continuous
☐ Rotational
☐ Intensive
☐ Combination (see question 7b)

b. If a combination of grazing management systems are used what **percentage** of your **cow-calf herd** is typically managed on each grazing system during the summer grazing season?
(If a particular type of pasture management is not used, please write 0%.)

Continuous _____%

Rotational _____%

Intensive _____%

7. Summer grazing system 2015 for cows:

a. During the summer grazing period of 2015 which of the following grazing systems would **best** describe the grazing system for your **cow-calf pairs** for the spring/summer pasture-grazing period?

(*Different grazing systems are described here. Please check the grazing system that* ***best*** *characterizes your grazing management for this management group.*)

Continuous grazing **-** The cattle have free range and determine which areas of the entire pasture available to them they will graze.
Rotational grazing **-** You move cattle through different pastures types but animal distribution is not directly managed. This grazing system utilizes larger areas, grazed for longer durations in rotation.
Intensive grazing **-** You determine where, when and what livestock graze at a set stocking rate and directly control animal distribution and movement, utilizing small areas usually grazed for short durations (i.e. 1 week.) and in the same season going back onto the same pasture.

☐ Continuous
☐ Rotational
☐ Intensive
☐ Combination (see question 7b)

b. If a combination of grazing management systems are used what **percentage** of your **cow-calf herd** is typically managed on each grazing system during the summer grazing season?
(If a particular type of pasture management is not used, please write 0%.)

Continuous _____%

Rotational _____%

Intensive _____%

8. Summer grazing system 2015 for replacement heifers:

a. During the summer grazing period of 2015 which of the following grazing systems would **best** describe the grazing system for your **replacement heifers** for the spring/summer pasture-grazing period?

(*Different grazing systems are described here. Please check the grazing system that **best** characterizes your grazing management for this management group.*)

Continuous grazing **-** The cattle have free range and determine which areas of the entire pasture available to them they will graze.
Rotational grazing **-** You move cattle through different pastures types but animal distribution is not directly managed. This grazing system utilizes larger areas, grazed for longer durations in rotation.
Intensive grazing **-** You determine where, when and what livestock graze at a set stocking rate and directly control animal distribution and movement, utilizing small areas usually grazed for short durations (i.e. 1 week.) and in the same season going back onto the same pasture.

☐ Continuous
☐ Rotational
☐ Intensive
☐ Combination (see question 9b)

b. If a combination of grazing management systems are used what **percentage** of your replacement **heifers** are typically managed as each grazing system during the summer grazing season?
(If a particular type of pasture management is not used, please write 0%.)

Continuous _____%

Rotational _____%

Intensive _____%

9. In the summer grazing season of 2015 did you utilize a community pasture for your **cow-calf herd**?

☐ Yes
☐ No

10. If a community pasture was utilized, approximately how many other herds was your cow-calf herd exposed to?

(If no community pasture was used, please write zero.)

Number of herds: _____

11. If a community pasture was utilized, how many of your **cow-calf pairs** were grazed on community pastures in the spring/summer pasture-grazing period of 2015?

(If no community pasture was used, please write zero.)

Number of cow-calf pairs: _____

12. During the **first 2 months** of your spring/summer pasture-grazing period of 2015, how would you classify your stocking density for your **cow-calf herd**?

(Just give us your best estimate. If stocking density is very different for each management group please answer for the largest management group of cow-calf pairs.)

☐ Intensively stocked (1 or more **cow-calf pairs**/acre)
☐ Moderately stocked (0.5-1 **cow-calf pairs**/acre)
☐ Lightly stocked (less 0.5 **cow-calf pairs**/acre)

13. During the **first 2 months** of your spring/summer pasture-grazing period of 2015, how would you classify your stocking density for your **replacement heifers** (heifers from weaning to 2 years of age kept for breeding purposes)?

(Just give us your best estimate. If stocking density is very different for each management group please answer for the largest management group of replacement heifers.)

- ☐ Intensively stocked (1 or more **heifers**/acre)
- ☐ Moderately stocked (0.5-1 **heifers**/acre)
- ☐ Lightly stocked (less 0.5 **heifers**/acre)

14. In the first 2 months of the spring/summer pasture-grazing season of 2015 did you graze replacement heifers (*heifers from weaning to 2 years of age kept for breeding purposes)* with mature cows?

- ☐ Yes
- ☐ No

15. In the first 2 months of the spring/summer grazing period, did any of your cow-calf pairs and/or replacement heifers have direct access to surface water?
(For example sloughs and dugouts)

	Yes	**No**
Cow-calf pairs	☐	☐
Replacement heifers	☐	☐

Section 2: Internal parasite control

16. For each class of animal groups listed in the table below please list the parasite control products applied from May 2014 to May 2016?

(If no parasite control products have been used in the last 2 years, please indicate this in the table below where appropriate. Please see the supplementary booklet for the list of registered parasite control products for beef cattle in Canada. If 'other' under route of administration, please specify in 'name of product used' box.)

a. MATURE COWS

Ever treated in the last 2 years	Route of administration (please check)	Name of the product used (please see supplementary booklet)	Date of administration (Please write month, eg. 30-May-16; if exact date unknown, please indicate nearest month/year)
☐ Yes ☐ No	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		

16. For each class of animal groups listed in the table below please list the parasite control products applied from May 2014 to May 2016?

(If no parasite control products have been used in the last 2 years, please indicate this in the table below where appropriate. Please see the supplementary booklet for the list of registered parasite control products for beef cattle in Canada. If 'other' under route of administration, please specify in 'name of product used' box.)

b. UN-WEANED CALVES

Ever treated in the last 2 years	Route of administration (please check)	Name of the product used (please see supplementary booklet)	Date of administration (Please write month, eg. 30-May-16; if exact date unknown, please indicate nearest month/year)
☐ Yes ☐ No	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ ☐ mineral mix ☐ Other		

16. For each class of animal groups listed in the table below please list the parasite control products applied from May 2014 to May 2016?

(If no parasite control products have been used in the last 2 years, please indicate this in the table below where appropriate. Please see the supplementary booklet for the list of registered parasite control products for beef cattle in Canada. If 'other' under route of administration, please specify in 'name of product used' box.)

c. REPLACEMENT HEIFERS (heifers from weaning to 2 years of age kept for breeding purposes)

Ever treated in the last 2 years	Route of administration (please check)	Name of the product used (please see supplementary booklet)	Date of administration (Please write month, eg. 30-May-16; if exact date unknown, please indicate nearest month/year)
☐ Yes ☐ No	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		
	☐ Drench ☐ Pour-on ☐ Injection ☐ In feed/ mineral mix ☐ Other		

17. If you used a parasite control product, how was the dose of the product to be administered determined for treated animals?

- ☐ Based on each animal's weight by a weigh scale or a weight tape
- ☐ Based on each animal's weight as determined by looking at the animal
- ☐ Other, please explain: _____
- ☐ I do not use a parasite control product

18. What is your **most important** source of information for parasite control?

- ☐ Personal experience
- ☐ Veterinarian
- ☐ Product representative
- ☐ Neighbour/ Other producer
- ☐ Other, please explain:_____

_____

19. What is your **most important** reason for choosing to use a parasite control product (please check only one)?

- ☐ Control of external parasites (lice, flies etc.)
- ☐ Control of internal parasites (worms)
- ☐ Symptoms observed (poor weight gain, diarrhea, rough hair coat etc.)
- ☐ Vet recommendation
- ☐ Neighbours are treating
- ☐ Heard parasites were a problem
- ☐ Climate/Weather
- ☐ Regular and routine practice on farm
- ☐ Other, please explain:_____

_____

20. For each factor below, please indicate how important it is to you when choosing a parasite control product:

a. Price:	☐ Not Important	☐ Important	☐ Very Important
b. How well it works on internal parasites (worms):	☐ Not Important	☐ Important	☐ Very Important
c. How well it works on external parasites (lice, flies etc.):	☐ Not Important	☐ Important	☐ Very Important
d. Ease of application:	☐ Not Important	☐ Important	☐ Very Important

21. In the last 3 years, have you or your vet taken fecal samples to test for worms?

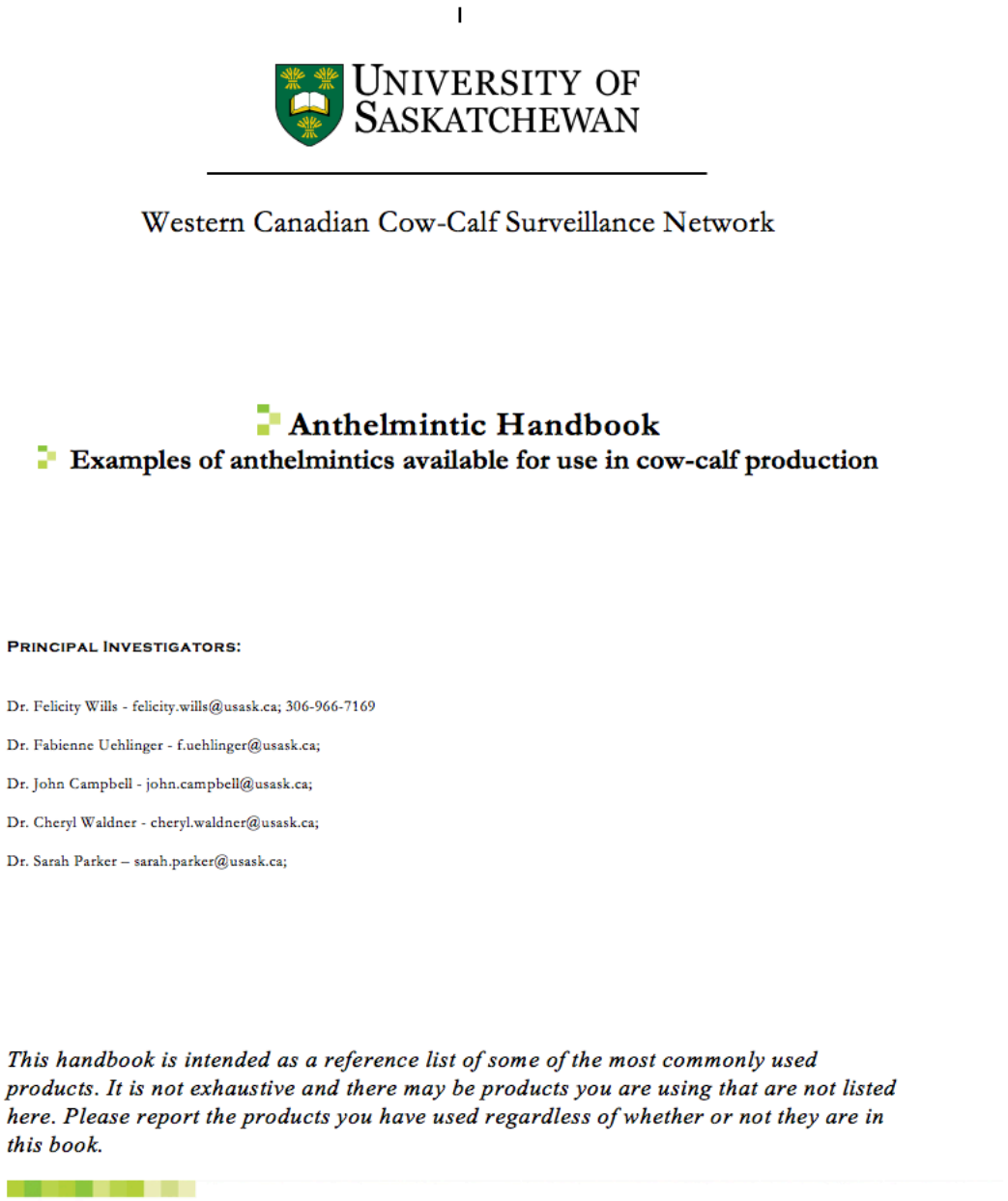
- ☐ Yes
- ☐ No
- ☐ Do not know

22. If testing was performed what groups of cattle were tested? (*Check all that apply.*)

- ☐ Un-weaned calves
- ☐ Replacement heifers (weaned to 2 years old but not calved)
- ☐ Steers (weaned or older)
- ☐ Cows (including heifers that had calved)
- ☐ Bulls (for breeding or sale)
- ☐ No testing performed

23. Do you have any other comments? (Please feel free to continue onto next page if needed.)

FIGURE A4.2 Western Canadian Cow-Calf Surveillance Network Parasite Control Product Handbook.



UNIVERSITY OF SASKATCHEWAN

Western Canadian Cow-Calf Surveillance Network

Anthelmintic Handbook

Examples of anthelmintics available for use in cow-calf production

PRINCIPAL INVESTIGATORS:

Dr. Felicity Wills - felicity.wills@usask.ca; 306-966-7169

Dr. Fabienne Uehlinger - f.uehlinger@usask.ca;

Dr. John Campbell - john.campbell@usask.ca;

Dr. Cheryl Waldner - cheryl.waldner@usask.ca;

Dr. Sarah Parker – sarah.parker@usask.ca;

This handbook is intended as a reference list of some of the most commonly used products. It is not exhaustive and there may be products you are using that are not listed here. Please report the products you have used regardless of whether or not they are in this book.

Table of Contents

1. Injectable Anthelmintics

Bimectin Injection
Ivermectin 10mg/ml

Cydectin Injectable Solution for Beef and Nonlactating Dairy Cattle
Moxidectin 10mg/ml

Dectomax Injection
Doromectin
10mg/ml

Ivomec Injection
Ivermectin 10mg/ml

Noromectin Antiparasitic Injection For Cattle And Pigs
Ivermectin
10mg/ml

Unimectin Injection
Ivermectin 10mg/ml

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2. Pour On Anthelmintics

Bimectin Pour- On For Cattle
Ivermectin 5mg/ml

Cydection Pour-On for Beef and Dairy Cattle
Moxidectin 5mg/ml

Dectomax Pour-On
Doromectin 5mg/ml

Eprinex Pour-On
Eprinomectin 5mg/ml

Ivomec Pour-On For Cattle
Ivermectin 5mg/ml

Ivermectin Pour-On For Cattle
Ivermectin 5mg/ml

Noromectin Pour-On For Cattle

Ivermectin 5mg/ml

Unimectin Pour-on

Ivermectin 5mg/ml

No photo available

3. Oral Anthelmintics

Panacur Dewormer For Beef and Dairy Cattle 10% Suspension
Fenbendazole 100mg/ml

Safe-guard Cattle Dewormer 10%
Fenbendazole 100mg/ml

Valbazen Broad-Spectrum Dewormer
Albendazole 113.6mg/ml

3. In feed products

SAFE-GUARD® 20% Salt: free-choice mineral (beef and dairy cattle)
Fenbendazole 2.27 gram per pound

No photo available

SAFE-GUARD® Medicated dewormer for beef cattle (20% protein block)
Fenbendazole 2.27 gram per pound

No photo available

SAFE-GUARD® Medicated dewormer for beef cattle (en-pro-al® molasses block)
Fenbendazole 750mg per pound

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SAFE-GUARD® Medicated dewormer for beef & dairy cattle (soft mini-pellets)
Fenbendazole 19.6 grams per kilogram

No photo available

SAFE-GUARD® Medicated dewormer for beef and dairy cattle (flaked meal)
Fenbendazole 19.6 grams per kilogram

No photo available

SAFE-GUARD® Medicated dewormer for beef & dairy cattle, & swine (0.5% top dress pellets 10 lbs)
Fenbendazole 2.27 gram per pound

No photo available